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„Carbon and nitrogen exchange between plant roots, ectomycorrhizal fungi and soil bacteria in the mycorrhizosphere of *Fagus sylvatica*"

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1. GENERAL INTRODUCTION

On the ecology of mycorrhizal associations with special emphasis on ectomycorrhiza

Almost all plants form mycorrhizae, which are belowground associations of plant roots with mycorrhizal fungi. The mycorrhizal symbiosis is widespread, covering almost all known terrestrial habitats. 80 % of land plant species, and 92 % of land plant families are thought to be mycorrhizal (Wang & Qiu 2006). Global distribution is ubiquitous, ranging from arctic and alpine tundra (Väre et al. 1992, Gardes & Dahlberg 1996), boreal forests (Read et al. 2004), temperate forests (Malloch et al. 1980, Taylor et al. 2000) and grasslands (Johnson et al. 2002, Escudero & Mendoza 2005), deserts (Miller 1979, Mejstřík & Cudlín 1983, Shi et al. 2006), to tropical rainforests (Husband et al. 2002, Zhao et al. 2003, McGuire 2007).
So far, four main types of mycorrhizae have been described: Arbuscular mycorrhiza (AM), ectomycorrhiza (ECM), ericoid and orchid mycorrhiza (Smith & Read 2008, van der Heijden et al. 2015). Mycorrhizae form intricate interfaces at roots, where fungal hyphae either penetrate root cells, forming intracellular arbuscules or coils (AM, orchid and ericoid mycorrhizal fungi), or grow inbetween roots cells (ECM fungi). The basis of the symbiotic relationship is the exchange of photosynthetically acquired carbon (C) from the plant host against nutrients and water provided by mycorrhizal fungi. Extraradical mycorrhizal hyphae reach soil areas outside the direct influence of roots, with greater length and smaller diameter than root hairs.
In this regard, mycorrhizal hyphae may be imagined as extended roots, increasing the surface area for water and nutrient uptake and the number of soil microsites reached by a plant dramatically, which has profound implications for plant growth and fitness, and ecosystem functioning. Indeed, mycorrhiza have large implications on ecosystem processes, with contributions to plant productivity and decomposition, plant N and P uptake, denitrification, species diversity, soil aggregation and seedling survival (van der Heijden et al. 2015).

1.1 Arbuscular mycorrhizal fungi

AM fungi are endomycorrhiza, meaning that fungal hyphae grow inside cortical cells of their plant host root. The name ‘arbuscular’ stems from the tree-like structures formed by hyphae penetrating root cells, called arbuscules. AM are the most ancient of mycorrhizal relationships, with fossil evidence dating up to 400 million years ago (Remy et al. 1994, Taylor et al. 1995). All land plants are believed to have initially evolved within an AM symbiosis (Wang & Qiu 2006, Cairney 2000). AM are the most common mycorrhizal type, occurring in most angiosperms and pteridophytes, as well as in some gymnosperms, lycopods and mosses (Smith & Read 2008). Estimates suggest that 73 % of all vascular plant species are involved in this symbiosis (Brundrett 2009). All AM fungi are of the phylum Glomeromycota, with estimates of total species numbers ranging between 300 to 1600 (van der Heijden et al. 2015, after Öpik et al. 2013 and Kivlin et al. 2011). They are obligate symbionts with their plant hosts and reproduce asexually (Schüßler et al. 2001). Due to this low fungal species/host species ratio, and since individual plant hosts are commonly associated with several fungal partners, it is generally assumed that AM fungi are not host specific (Klironomos 2000, Smith & Read 2008). However, Chanway et al. (1991) suggest that, as most observations are made under controlled laboratory or greenhouse conditions with a limited number of organisms, host-specificity of AM fungi may be more common in nature where plant hosts are exposed to a highly diverse soil microbial community.
AM fungi are associated with monocots and non-woody plants, but also form mycorrhiza with large trees. They are predominantly found in grassland ecosys-

tems, as well as forests in tropical and subtropical biomes (Smith & Read 2008). As such, they seem to preferentially occur in species-rich ecosystems. However, some typical taxa of temperate forests like *Acer*, *Fraxinus* and *Ulmus*, also form AM (Eissenstadt et al. 2015). In a recent study providing a global dataset on mycorrhizal plant root colonization, Soudzilovskaia et al. (2015) showed that AM fungi prefer climates with warm-season temperatures and frost periods, and low C/N ratios in soils.

1.2 Ectomycorrhizal fungi

The defining characteristic of ECM associations is the development of ectomycorrhizal root tips (Fig. 1.1), representing the symbiotic interface. These are composed of two structural components: an outer mantle of fungal mycelium which encapsulates the root, and fungal hyphae growing between epidermal and cortical cells without ability to pass the Casparian strip, called Hartig net (Smith & Read 2008; Fig. 1.2). In contrast to AM fungi, ECM hyphae do not penetrate host cells (hence the prefix '-ecto'). From root-tips as a starting point, extraradical hyphae grow into distant soil areas, gaining access to nutrients and water in soil beyond the direct influence of plant roots. Development and differentiation of the extraradical mycelium reflects ecological features of the fungus and thus potentially predicts its occupied niche. Agerer (2001) differentiates between several types of ECM hyphae based on the morphology and development of extraradical hyphae, namely contact, short-, medium- and long-distance types. Rhizomorphs are a defining feature of long-distance exploration types. Regardless of the exploration type of an ECM fungus, its most distant hyphae are on average much thinner than other hyphae, appear to be hydrophilic, and are most likely responsible for the uptake of nutrients and water (Agerer 2001 and references therein).

Contrary to AM fungi, ECM fungi are highly diverse. Conservative estimates on ECM diversity range from 5.000–8.000 fungal species (Molina et al. 1992, Rinaldi et al. 2007), but extrapolations predict 20.000–25.000 (Rinaldi et al. 2007). These are associated with ca. 6.000 plant species, or 4.5 % of terrestrial plant species (Brundrett 2009). Therefore, contrary to AM, ECM show a high fungal species/host species ratio. ECM plants and fungi are thought to generally show low host-specificity, both being capable of establishing the symbiosis with multiple partners. For instance, western hemlock (*Tsuga heterophylla*) forms ECM with more than 100 fungal species (Kropp & Trappe 1982), and *Boletus edulis* associates with at least 22 plant host species of Pinaceae, Betulaceae, Juglandaceae, Fagaceae, Oleaceae, Platanaceae and Salicaceae (Trappe 1962). Some ECM fungal species, however, only form mycorrhiza with a specific species, or species within a specific genus (Molina & Trappe 1982). ECM fungi predominantly belong to the phyla Basidiomycota and Ascomycota, but are also formed by some Zygomycota (Tedersoo et al. 2010). Indeed, fruiting bodies of ECM fungi are of value to human culture and trade, including archetypical and economically important mushrooms like *Amanita*, *Boletus*, *Tuber* and *Cantharellus*.

ECM are found predominantly in trees and woody shrubs of forests in temperate and boreal biomes, and to some extent in tropical ecosystems (Smith & Read 2008, Tedersoo et al. 2012). Even though ECM are formed with a relatively small number of plant species, their global ecological importance is prevailed by their association with Pinaceae and Fagaceae, members of which form vast monodomi 2008, Tedersoo et al. 2012). Even though ECM are formed with a relatively small

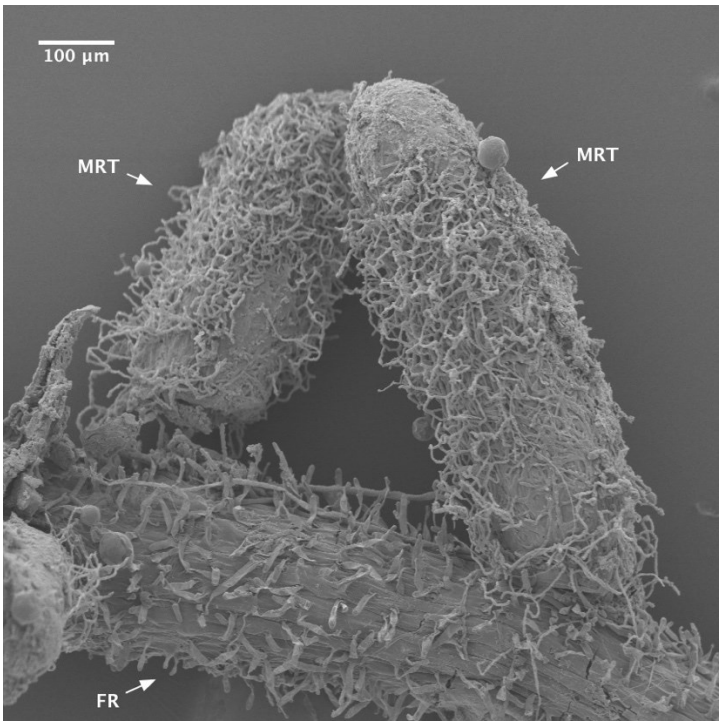

Figure 1.1 SEM image of mycorrhizal root tips of *Fagus sylvatica*. MRT, mycorrhizal root tip with hyphal mantle; FR, fine root with root hairs. Image kindly provided by Raphael Gabriel.

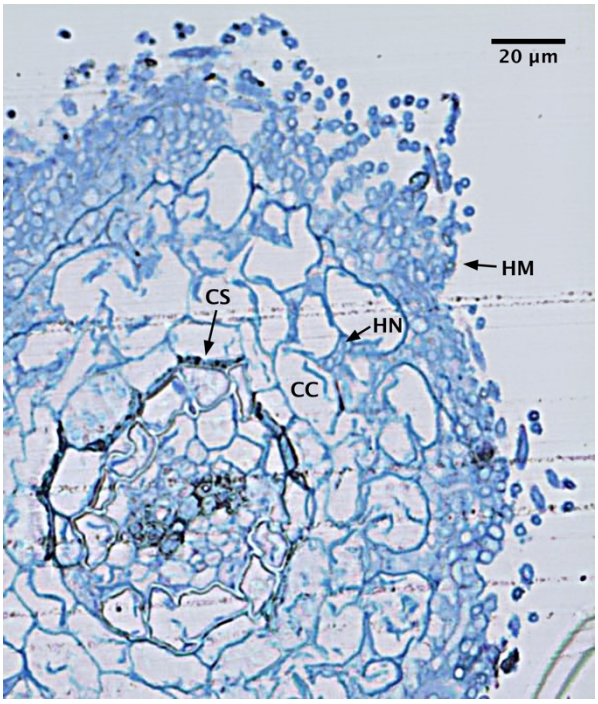

Figure 1.2 Light microscopic cross section of an ectomycorrhizal root tip of *Fagus sylvatica*, close to the root cap. Mantle hyphae are clearly distinguishable. HM, hyphal mantle; CC, cortical cell of plant root; HN, hartig net; CS, casparian strip. Image kindly provided by Werner Mayerhofer.

number of plant species, their global ecological importance is prevailed by their association with Pinaceae and Fagaceae, members of which form vast monodominant forests in boreal and temperate regions of the northern hemisphere. ECM occurrence is associated to acidic soils with high C/N ratios (Soudzilovskaia et al. 2015), where nitrogen (N) is the overall growth-limiting nutrient for plants.
Overall, a total of at least 66 distinct evolutionary origins have been suggested for the ECM lifestyle (Tedersoo et al. 2010), descending from ecologically diverse guilds as brown-rot and white-rot fungi, and other free-living saprotrophs. ECM fungi from the order Agaricales alone evolved from saprotrophic ancestors at least 11 times (Matheny et al. 2017). The shift from a saprotrophic to a biotrophic lifestyle has resulted in a significant loss of enzymatic potential to degrade plant cell walls, lignocellulose or recalcitrant humic complexes (Martin et al. 2016, Lindahl & Tunlid 2015). However, ECM fungi are functionally diverse, having not only lost but also retained enzymatic capabilities depending on their evolutionary history. For example, genes encoding class II peroxidases responsible for lignin decomposition, are convergently lost in ECM taxa. However, the ability to produce class II peroxidases is retained in Agaricales genera like *Cortinarius*, *Russula* and *Lactarius* (Bödeker 2009, 2014).

1.3 Reciprocity in the mycorrhizal symbiosis

The mycorrhizal relationship between plants and fungi has a long evolutionary history. Therefore, the symbiosis can be said to be evolutionary stable. However, mechanisms governing the stability of the symbiosis are poorly understood.
Bidirectional exchange of resources within ECM has been shown repeatedly (Finlay & Read 1986, Leake et al. 2001, Valtanen et al. 2014, Pickles et al. 2016, Finlay et al. 1988, 1989). The principal terms of trade are suggested as follows: C, which is available in excess for plants, is exchanged for N or other nutrients offered by ECM fungi. In ECM dominated ecosystems, N is the overall limiting nutrient for plant growth. However, benefits to the plant are not limited to increased N acquisition. Apart from N, fungi also provide phosphorus and other nutrients (in fact, in AM dominated ecosystems, phosphorus is the main limiting nutrient), increase their host plants resistance to drought, heavy metals and stress, and protect it against pathogen infection (Marx 1972, Newsham et al. 1995). Photosynthetically acquired C provided by their host plant is important for ECM fungi not only as their major C source, but also as a means for increasing access to soil nutrients. While ECM fungi have limited capacity to decompose organic matter compared to saprotrophic fungi, they can however release additional C input to stimulate activities of free living soil saprotrophs, which liberate nutrients from complex organic matter.
The interactions between plant and fungi are not necessarily mutualistic. For instance, fungi might withhold recourses, while still receiving photosynthates at the expense of the plant. In turn, such cheaters could be punished by the plant by reducing the C flow directed towards these fungi. Therefore, whether exchange of C for nutrients is reciprocal (i.e. providing or withholding resources is rewarded or punished, respectively), or if so, at what scale the exchange is controlled, remain major outstanding questions. Direct evidence for bidirectional control in the AM symbiosis was found *in vitro* with root organ cultures (Kiers et al. 2011). They showed that roots preferentially cooperate with hyphae which give more phosphorus, and in turn hyphae reward roots which give more C.

As trees are typically associated with several fungi, and ECM fungi may connect to several, phylogenetically distant trees, the ECM symbiosis encompasses a multi-agent system with potential actions between numerous partners. In this light, the evident evolutionary stability seems paradoxical indeed, because individuals can increase their net benefit by reducing their cost (i.e. defecting instead of cooperating). The capacity to distinguish and punish such partners becomes increasingly difficult when simultaneously interacting with multiple partners, even supposing that cooperation can be favoured through positive feedback mechanisms when both partners repeatedly interact. Concentrating on interactions between two partners, choices are limited to either cooperation, defection, or no interaction at all. However, interactions between several partners would provide a fourth possibility, that is, switching to a partner that is more beneficial. Borrowing terminology from economic sciences, biological market theory implies that when several partners interact, market selection favours the maximisation of fitness of the overall trading system (Noë & Hammerstein 1994, 1995, Hammerstein & Noë 2016). So long as overall net benefits in trade persist, the occurrence of cheating mechanisms is intrinsic to biological markets. In this framework, environmental changes may feed back to plant choices which fungal partner to establish a relationship with (in the case of plant control on partner choice), or to cooperate with (in the case of plant control on C allocation based on cooperation).

In this regard, associating with multiple partners is a conceivably viable strategy to increase the overall stability of the symbiosis after all, so long as partner choice is possible. Since fungal partners vary in their potential metabolic capacity to provide resources and services for the plant, a range of partners potentially widens the tolerance for environmental perturbations. For example, Pena et al. (2013) find that short- to medium- and medium-distance exploration type species harvest N from leaf litter, while contact, short-distance and long-distance exploration types use other sources. Refraining from the phyto-centric view which pervades most literature, the same strategy is potentially used by mycorrhizal fungi, as they associate with multiple host plants (e.g. Wu et al. 2001, Song et al. 2014, Pickles et al. 2016), often from different species (Hay et al. 1996, Graves et al. 1997, Simard et al. 1997, McKendrick et al. 2000). Thus, mycorrhizal fungi have access to multiple trading partners as well, possibly discriminating for partners sharing most photosynthates, and certainly gaining increased resistance to environmental perturbations because continuous C supply is maintained, even when one plant has restricted photosynthetic power (e.g. by herbivore damage). Such common mycorrhizal networks are thought to be the standard situation in most terrestrial ecosystems.

With their ‘luxury resource exchange’ hypothesis, Kiers & van der Heijden (2006) suggest that when both C and N are available in excess, the symbiosis is stable. This means that plants increase C allocation to mycorrhizal fungi with increased photosynthesis, because shifts in stoichiometric requirements change the plants allocation behaviour towards gaining the most limiting resource. Indeed, photosynthesis is sink-limited (Fatichi et al. 2003), and low belowground allocation and consequent accumulation of C in plant tissues has been suggested to down-regulate photosynthesis (Fitter 1991). Moreover, photosynthetic capacity correlates with mycorrhizal colonization (Hobbie 2006), suggesting preferential investment of non-limiting resources towards gaining advantages by improving availability of limiting resources.

1.4 Mycorrhizal ecosystem functioning

ECM fungi are strong sinks for photosynthetically acquired C. Between 14 and 22 % of total plant C uptake are allocated to ECM hyphae (Hobbie 2006). As such, large fractions of ecosystem net primary production is rapidly allocated into belowground soil pools. For example, in a tree-girdling experiment in a boreal forest, Högberg et al. (2001) demonstrated that a large fraction of soil respiration is derived from recent photosynthates. 1-2 months after girdling, soil respiration was reduced by 54 %, attributed to loss of activity by ECM mycelium.

How much C is then retained in ECM hyphae, however, remains elusive. Estimation of ECM fungal biomass is difficult, especially because of the methodological difficulty to distinguish between ECM and saprotrophic hyphae (Godbold et al. 2006). Calculating soil microbial biomass reduction from the girdling experiment mentioned above, Högberg & Högberg (2002) estimated that ECM extraradical mycelium contributes at least 32 % to the total soil microbial biomass, equivalent to 145 kg ha^{-1}. Using hyphal-in-growth bags, Wallander et al. (2001) distinguished ECM from saprotrophic fungi in boreal forest soil by filling the mesh bags with sand (which is depleted in organic matter), and measuring non-mycorrhizal controls. They estimated biomass ECM of extraradical mycelium in the humus layer via the fungal biomarkers ergosterol (700 kg h^{-1}) and phospholipid fatty acid 18:2ω6,9 (900 kg h^{-1}). With the same experimental procedure, Wallander et al. (2004) observed ECM fungal biomass of 4 to 6 t h^{-1} in spruce and spruce-oak forests in southern Sweden. Compared to fine root biomass, which has been estimated at 5.3 t h^{-1}, extraradical mycelium would represent a significant pool of C bound in living biomass in boreal forests. Effectively, ECM fungal biomass comprises a significant proportion of soil microbial biomass, and turnover of ECM hyphae represent a significant input into forest soil C and nutrient stocks. Turnover rate estimates range from once per week to several months (Ekblad et al. 2013). However, fast turnover rates are mostly based on laboratory studies which typically use monocultures, and may not be applicable to field conditions. Field studies show considerably lower turnover times. For example, Hendricks et al. (2016) found a turnover time of 10 $year^{-1}$ by assessing ergosterol content in hyphal ingrowth cores in a subtropical *Pinus palustris* plantation, and Wallander et al. (2004) even found ECM extraradical mycelium to fully turnover every 10 years. Decomposition of ECM hyphal necromass is thought to be negatively effected by the recalcitrant nature of chitin (Godbold et al. 2006, Langley & Hungate 2003). New findings, however, suggest that chitin does not limit decomposition, and may in fact influence it positively (Fernandez & Koide 2012). Other intrinsic factors like melanin concentration and N content probably exert a major fraction of control on decomposition rates of ECM hyphal necromass (Fernandez et al. 2016).

Even though ECM fungi lost saprotrophic capabilities in their evolutionary history to a certain degree, they are able to decompose organic matter by production of extracellular enzymes (Durall et al. 1994, Bending & Read 1995, Colpaert & van Tichelen 1996, Shah et al. 2016, Talbot et al. 2008, Lindahl & Tunlid 2015). Hence, they partly retained the ability to release nutrients from recalcitrant molecules. In a simplistic experiment, Gadgil & Gadgil (1971) observed that litter decomposition is significantly enhanced when excluding mycorrhizal fungi. They attributed this effect to saprotrophic organisms being relieved of suppressive interactions with mycorrhizal fungi. The literature fittingly refers to competitive interactions of ECM and saprotrophic fungi and consequent suppression of de-

composition as the 'Gadgil effect'. Even though ECM fungi degrade SOM, they are not saprotrophs, because a saprotrophic lifestyle implies that C received from complex organic compounds is the primary source of C, while ECM fungi receive most of their C from their host plants (Talbot et al. 2008). Therefore, ECM fungi are thought to have a competitive advantage against saprotrophs in soil areas with high C/N ratios, suggesting spatial separation across soil horizons of the two fungal guilds. In fact, ECM fungi have been observed to predominantly occur in decomposed litter and humus (Baier et al. 2006, Lindahl et al. 2007, McGuire et al. 2013), while saprotrophic fungi prefer recent plant litter. Indeed, mining for N in SOM by ECM hyphae might create a positive feedback loop, where only a fraction of C is respired, resulting in accumulation of C in SOM. Lindahl and Tunlid (2015) proposed that mycorrhizal fungi replace saprotrophic fungi at later stages of decomposition. Saprotrophic decomposition is driven via hydrolysis of lignocellulose, while ectomycorrhizal fungi have lost most of their enzymes acting on plant cell wall material, preferentially facilitating degradation of organic matter by exudation of oxidative enzymes.
Moreover, ECM fungi may be able to 'prime' soil saprotrophs by exuding labile C compounds, much like the priming effect observed in rhizosphere soil, to enhance decomposition by saprotrophic organisms (see paragraph 'Mycorrhizal interactions with soil bacteria'). This may lead to cooperation between ECM and saprotrophic fungi and bacteria. However, due to their larger surface/volume ratio, exuded C is likely taken up primarily by saprotrophic bacteria.

1.5 Mycorrhizal interactions with soil bacteria

Before forming a symbiotic relationship with a plant, mycorrhizal fungi have to grow through soil to find potential root surfaces. During this free-living stage in its ontogeny, the fungus encounters a variety of soil organisms. Bacteria which facilitate positive conditions for mycorrhization are termed 'mycorrhization helper bacteria'. They support spore germination, promote hyphal growth, and enhance soil conditions favourably for mycorrhizal establishment (Garbaye 1994, Frey-Klett et al. 2007), and consequently increase colonization rates on plant roots.
Frey-Klett et al. (2007) emphasise the importance of distinguishing between mycorrhiza*tion* and mycorrhiza helper bacteria. Mycorrhiza helper bacteria fulfil functions directly maintaining the established relationship between plant and fungus. For instance, bacteria detoxify soil (Duponnois & Garbaye 1990), influence mycelial growth (Duponnois & Garbaye 1990, Schrey et al. 2005), and N_2–fixing bacteria associated with mycorrhizal hyphae increase N availability for both mycorrhizal fungus and plant (Li & Hung 1987).
In addition, many soil saprotrophic bacteria thrive on recent photosynthates from the plant, resulting in accelerated decomposition, commonly referred to as the priming effect (Kuzyakov et al. 2000, Kuzyakov 2002, 2010). Priming effects are usually observed when adding (often ^{13}C labelled) simple sugars, amino and organic acids to soil, mimicking root exudates. The resulting acceleration of SOM decomposition is measured as increase in respiration rate of native (unlabelled) SOM. Traditionally, priming was theoretically limited to rhizosphere soil; modern literature diverges in theoretical distinctions on how to approach priming effects:

i. Plant roots exude labile C, which is metabolized by soil organisms, including mycorrhizal fungi. This view implicitly distinguishes between plant and soil organisms.
ii. Priming is the release of labile C into soil. As mycorrhizal hyphae receive C directly from the plant (without intermediate residence time in soil), this view recognizes symbiotic mycorrhizal partners as a unity. C is either exuded by non-mycorrhizal roots, or by mycorrhizal hyphae.

In (i), ECM fungi are by themselves seen as carrying structures of the priming effect (Talbot et al. 2008, Murphy et al. 2015), or analogous to it (Lindahl & Tunlid 2015). In this reasoning, the fungus is seen as part of the plant's rhizosphere, where plant-derived C primes the fungus, which subsequently returns N acquired from enzymatic SOM decomposition to the plant. To my mind, this approach has the potential to distort our view on soil processes as it (a) distinctly compares free living soil bacteria and mycorrhizal fungi exclusively from a plant perspective, (b) confuses functional components of soil systems which have different effects on C and nutrient cycling, and (c) undermines the general validity of the mycorrhizal symbiosis in terrestrial ecosystems. In (ii), plant and mycorrhizal fungi act as a unity, where fungal hyphae increase the soil area under influence of mycorrhizal plants. This approach is especially evident when collectively referring to mycorrhizosphere as the soil influenced by both plant roots and mycorrhizal hyphae, combining rhizosphere and hyphosphere (e.g. Johansson et al. 2004).
Considering the ubiquity of mycorrhizal associations, priming by recent photosynthates via mycorrhizal hyphae may be pervasive in terrestrial ecosystems, although not much is known about it yet. Indeed, priming by extraradical hyphae seems especially plausible with AM fungi, as they are not known to encompass enzymatic abilities to decompose SOM (Smith & Smith 2011, Jansa et al. 2013). For instance, Cheng et al. (2010) argue that an observed increase of decomposition rates in AM systems under elevated CO_2 is due to increased AM hyphal exudation rates. Further, AM hyphae were demonstrated to have a pronounced effect on soil bacterial community composition, likely by exuding low-molecular-weight compounds composed of formiate, acetate, glucose, a starch-like compound and di- and oligosaccharides (Toljander et al. 2007). By applying stable isotope labelling with ^{13}C-CO_2, plant-derived ^{13}C was found in bacteria-specific phospholipid fatty acids and RNA in AM systems (Drigo et al. 2010). Using a similar approach, Kaiser et al. (2014) observed transfer of recently photoassimilated C to bacteria-specific phospholipid fatty acids in soil exclusively accessible by AM hyphae, providing direct evidence of hyphospheric C allocation. To my knowledge, no experimental evidence for hyphospheric priming in other mycorrhizal types exists, although theoretical considerations suggest it to be likely. ECM fungi may maintain saprotrophic abilities, but would overall still rely on other free-living saprotrophs to provide enzymatic potential, and constant provision of C from their plant host makes C a dispensable resource. As a major outstanding question remains whether mycorrhiza-associated bacteria act as 'hypersymbionts' (Jansa et al. 2013), that is, whether they are metabolically coupled to mycorrhizal hyphae, that is, most of their C need is fulfilled by hyphal exudation, or if they mainly derive their C from mineralizing SOM.

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2. MANUSCRIPT

Carbon and nitrogen exchange between plant roots, ectomycorrhizal fungi and soil bacteria in the mycorrhizosphere of *Fagus sylvatica*

2.1 Introduction

The establishment of mycorrhiza has been a major evolutionary step in the Earth's history. This symbiosis between plants and fungi played a fundamental role in the colonisation of terrestrial habitats by plants (Brundrett 2002). Plants exchange carbon (C) for nutrients and water with associated mycorrhizal fungi, which has significant positive implications for plant fitness. The ubiquity of arbuscular mycorrhiza (AM) (73 % of all vascular plants are associated with this mycorrhizal type; Brundrett 2009), AM associations with bryophytes (Wang & Qiu 2006) and fossil evidence (Remy et al. 1994, Taylor et al. 1995) suggests that all land plants have initially evolved within an AM symbiosis. Ectomycorrhiza (ECM), on the contrary, evolved only later during the Jurassic and the Cretaceous in several independent lineages from saprotrophic fungal ancestors (Brundrett 2002, Cairney 2000, Tedersoo et al. 2010, Martin et al. 2016). This detachment from a free-living lifestyle resulted in loss of enzymatic capabilities to degrade soil organic matter (SOM). However, different lineages of ECM fungi have probably retained saprotrophic capacities at various extents (Bruns et al. 1998).

Reciprocity in the ectomycorrhizal symbiosis

Although the mycorrhizal symbiosis is generally considered beneficial for both plant and fungus, it encompasses a continuum of interactions ranging from mutualistic to neutral to parasitic (Johnson et al. 1997, Kiers & van der Heijden 2006). As individual plants form mycorrhizae with several fungal species (AM and ECM fungi generally show low host specificity), the possible space of relationships is open for both cooperators and cheaters, while still accounting for a positive net effect for the plant. However, the mechanisms of exchange of C and nutrients within single mycorrhiza are poorly understood, with recent research focusing on AM fungi. For example, Kiers et al. (2011) observed that individual roots allocate significantly more C to mycorrhizal partners that delivered more phosphorus (P) than others. Such a reciprocal reward would result in bidirectional control of both partners, and hence could be a potential mechanism to punish cheating partners as well as maintaining evolutionary stability of the symbiosis (Noë & Hammerstein 1995, Kiers & van der Heijden 2006). In contrast to AM research, literature on exchange mechanisms in ECM is scarce, with notable examples pointing towards low plant-control on C flux based on received N at longer timescales (Valtanen et al. 2014). In a short-time microcosm experiment, Hortal et al. (2017) found no correlation between plant C flux and fungal N provision; however, as they point out, their experimental setup was not N limited, reflecting poorly on natural conditions. Thus, whether the ECM symbiosis depends on reciprocal interactions between their partners remains a major outstanding question. If so, ECM plant roots may adjust C flux to fungi individually at the level of single root tips, or single mycorrhizal roots, or bulk roots depending on the net cooperation (i.e. nutrient delivery) of mycorrhizal fungi in a certain area of the root system.

Carbon allocation to soil bacteria via the ectomycorrhizal pathway

Terrestrial ecosystems occupy a fundamental role in the global C cycle. Acting as C sinks, they are responsible for sequestration of about a quarter of anthropogenic CO_2 thus far, with soil being the largest source of CO_2-reflux back to the atmosphere (Ciais et al. 2013). Overall, plants allocate considerable amounts of recently photoassimilated C belowground. Allocation pathways are either through mycor-

rhizal fungi (Leake et al. 2001, Simard et al. 2003, Johnson et al. 2002), or direct exudation into adjacent soil (Dilkes et al. 2004, Bahn et al. 2009). Transport of easily available, labile C compounds to soil via root exudates results in *rhizosphere priming*, that is the subsequent acceleration of SOM decomposition by heterotrophic soil microbes (Kuzyakov 2002). As bacteria are often energy-restricted in soil, additional low-molecular weight C compounds increase their metabolic capability to produce extracellular enzymes degrading high-molecular compounds, resulting in increased availability of labile organic and inorganic N and P to both bacteria and plant.

Mycorrhizal hyphae produce extensive extraradical mycelia (Agerer 2001), conceptually analogous to 'extended roots', which dramatically increase the plant's accessible soil volume. The classical distinction of rhizosphere soil as 'the soil influenced by roots' (Hiltner 1904) seems equivocal in this light: photosynthetically acquired C is transported readily to mycorrhizal hyphae, which in turn allocate it to soil areas out of reach for plant roots. Concurrently, extraradical hyphae allocate nutrients and water to plant roots, making resources from distant soil regions available for plants.

Much of past research has concentrated on beneficial effects of mycorrhiza on plants by acquiring *inorganic* nutrients and water. As a consequence, saprotrophic and mycorrhizal fungi emerged in the literature as functionally separate guilds occupying distinct niches, with free-living saprotrophs solely responsible for organic matter decomposition and consequent release of nutrients (Read & Perez-Moreno 2003). However, the validity of this reductionist functional distinction has been increasingly questioned. For instance, both AM and ECM fungi have been shown to utilize N from organic material (Hodge et al. 2001, Rineau et al. 2013). Furthermore, ECM fungi are known to produce a range of extracellular enzymes capable of degrading complex organic compounds (Chalot et al. 1996, Read et al. 2004, Talbot et al. 2008). Thus mycorrhizal and saprotrophic fungi occupy overlapping niches, competing for the same nutrients bound in complex organic matter. Accordingly, ECM fungi have been suggested to be in a competitive situation with saprotrophic fungi (Gadgil & Gadgil 1971, Fernandez & Kennedy 2015). However, ECM fungi are functionally diverse, and it is not fully clarified to what extent they retained saprotrophic capabilities from their ancestors (Bruns et al. 1998). Thus, although the provision of labile C from their host plants is advantageous, the exact mechanisms governing competition or cooperation with saprotrophic fungi and bacteria for nutrients in SOM are poorly understood.

Current hypotheses suggest that because AM fungi lack production of SOM degrading enzymes (Smith & Read 2008), they rely on associated saprotrophic microorganisms for access to nutrients bound in organic matter (Hodge et al. 2010, Cheng et al. 2012, Jansa et al. 2013). Transfer of plant-derived C to bacteria via AM hyphae is supported through indirect (Toljander et al. 2007, Drigo et al. 2010, Cheng et al. 2012, Paterson et al. 2016) and direct evidence (Kaiser et al. 2014). Thus, *hyphosphere priming* with plant photosynthates may be a potentially important process in C cycling in AM-dominated ecosystems (Talbot et al. 2008). In contrast, the diverse enzymatic capabilities of ECM fungi for N acquisition from organic matter add complexity to their relationship with soil bacteria. While AM fungi have been shown to allocate C to soil bacteria, hyphospheric interactions of ECM fungi remain ambiguous.

Here, we examined C and N flow through an ECM system with the following research questions: (i) Is there a reciprocal exchange of C and N at the highest

order of the root system architecture, i.e. if part of the root system receives more N, is there also more recently photoassimilated C allocated into this part? (ii) Do ECM fungi transfer plant-derived C to associated hyphosphere bacteria? If so, (iii) how do ECM fungi and soil bacteria respond to increased availability of easily accessible N.

To address these questions, we conducted a dual stable isotope labelling experiment with young *Fagus sylvatica* trees, exposing the aboveground plants to a ^{13}C-CO_2 enriched atmosphere, and adding ^{15}N labelled ammonium and amino acids to a root-inaccessible compartment, to which only mycorrhizal hyphae associated with only a part of the root system had exclusive access. We subsequently compared ^{15}N-amended and untreated root system parts regarding their ^{13}C allocation, and traced the fate of recently photoassimilated ^{13}C into soil bacteria via phospholipid fatty acid stable isotope probing and nanoscale secondary ion mass spectrometry (NanoSIMS).

2.2 Material and methods

Experimental setup

Three to four year old *Fagus sylvatica* L. trees were collected in the Vienna Woods forest area near Vienna. They were planted in boxes consisting of two soil compartments (each 114 × 60 × 125 cm), dividing the root system in half. Mycorrhization was confirmed on all plants. Each soil compartment was connected to a litter compartment (10 × 60 × 125 cm), separated by a mesh (35 µm) which is penetrable by fungal hyphae, but acts as a barrier to plant roots (Fig. 2.1). Both soil and plant litter were native to the collection site. Plants were left in this setting for 12 months in a glasshouse under natural sunlight, temperature, and otherwise controlled conditions, where a movable ceiling stays open unless rain or strong winds are registered; watering was done by hand.

Fourty hours before sampling, a ^{15}N-labelled solution was added to left litter compartments only (12 ml of 1 mM ^{15}N-labelled NH_4 and amino acid mix). To ensure equivalent conditions, tap water was added to right litter compartments. Diffusion was prevented (i) between the soil and litter compartments by the structure of the mesh, which consisted of two membranes with an intermediate air-filled space, and (ii) between plant boxes by resting them on wooden blocks, preventing water transfer.

24 h after ^{15}N–addition, aboveground plant parts were incubated in a ^{13}C-CO_2 enriched atmosphere (approximately 90 atom% ^{13}C; 1500 ppm) for 6 h. This was done with a plexiglass incubation chamber (Fig. 2.2). Plant stems were fitted through small holes at the bottom of the chamber, so that aboveground plant parts were exposed to chamber atmosphere. Circa one half of the stem reached inside the chamber, and the hole was sealed with Terostat® adhesive. Thus, plant canopies were exclusively exposed to ^{13}C-CO_2, while soil and litter compartments stayed outside the labelling chamber. Additionally, photosynthesis in plant boxes was prevented by isolating boxes with black plastic bags. Constant measurement of ^{13}C-CO_2 concentration with an IRGA system verified significant enrichment of chamber atmosphere.

After the ^{13}C-CO_2 labelling, plants were kept overnight at a cool place in the dark and harvested during the next day (11-18 h after the end of the labelling period), distinguishing between the pools *leaves*, *stem*, *roots*, *rhizosphere* and *bulk soil* (soil-compartment exclusive) and *litter* (litter-compartment exclusive). Bulk soil was defined as the soil which stayed inside the box after removing the plants, including soil which falls off the roots when shaking the out-of-box plant lightly. Consequently, rhizosphere soil was defined as the soil which stays attached to the roots after light shaking. Each root-system half (left and right) was separated into three to four parts for further processing based on root system architecture (that is, fourth- to fifth-order roots), with differentiation between coarse and fine roots.

A total of eight plants received ^{15}N– and ^{13}C-CO_2–treatment, four plants were unlabelled controls, and four unlabelled boxes were kept without plants.

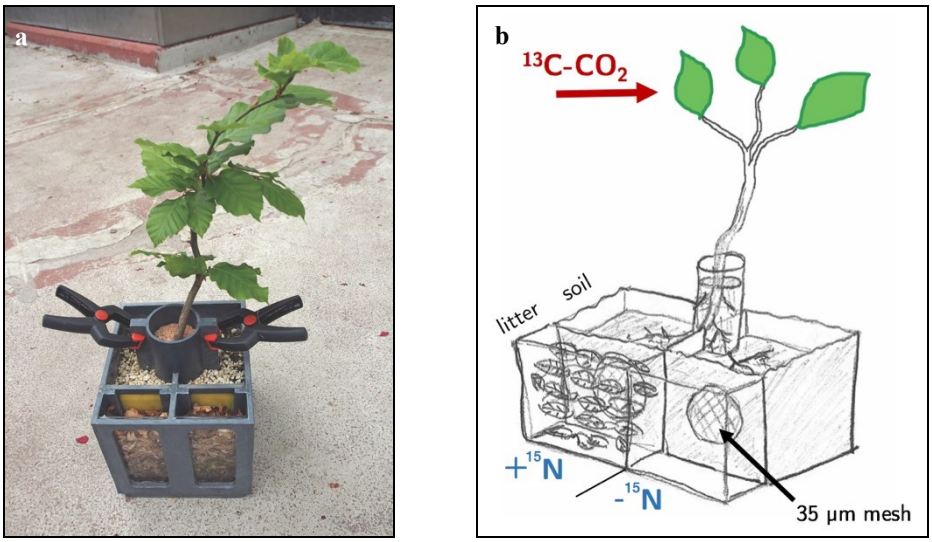

Figure 2.1 Design of split–root boxes. (a) Picture of box with *F. sylvatica* tree shortly before labelling with stable isotopes. Perlite was also applied on top of the soil compartment to prevent establishment of other plants. (b) Sketch depicting experimental setup with split-root box consisting of each two mesh-connected soil and litter compartments.

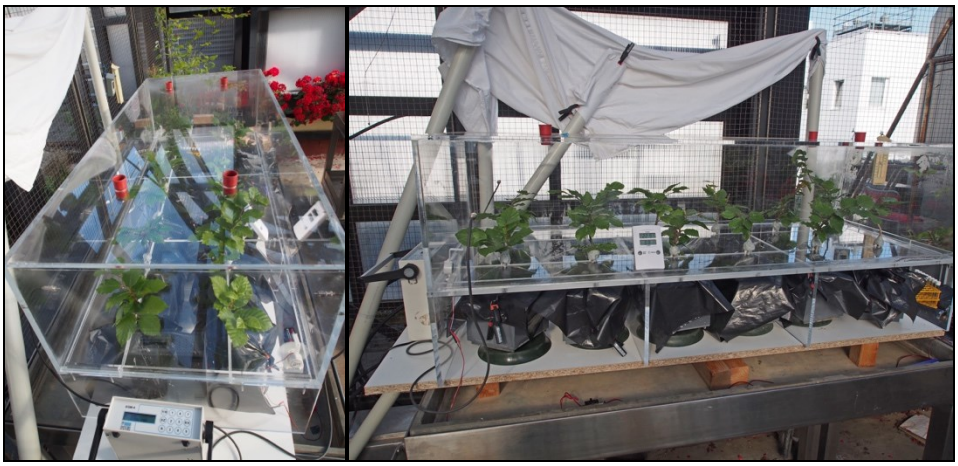

Figure 2.2 Labelling *F. sylvatica* plants in the incubation chamber.

Tracing plant-derived carbon and hyphal-assimilated nitrogen (EA-IRMS)
Allocation of recently photoassimilated ^{13}C and added ^{15}N in bulk pools was measured via isotope ratio mass spectrometry coupled to an elemental analyser (EA-IRMS; CE Instrument EA 1110 elemental analyzer, coupled to a Finnigan 141 MAT DeltaPlus IRMS with a Finnigan MAT ConFlo II Interface). Isotopic abundance was measured as

$$\delta\,{}^{heavy}I = \left(\frac{{}^{heavy}I_{sample} \,/\, {}^{light}I_{sample}}{{}^{heavy}I_{standard} \,/\, {}^{light}I_{standard}} \right) \times 1000‰$$

as well as

$$atom\%\ {}^{heavy}I = \left(\frac{{}^{heavy}I}{{}^{light}I + {}^{heavy}I} \right) \times 100$$

where $^{heavy}I$ and $^{light}I$ are heavy (^{13}C, ^{15}N) and light (^{12}C, ^{14}N) isotopes, respectively. Reference standards used were Vienna Pee-Dee Belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$. Abundance of ^{13}C as exclusively recent photosynthates, and added ^{15}N, was calculated as

$$atom\%\ excess\ {}^{13}C_x = atom\%\ {}^{13}C_x - atom\%\ {}^{13}C_{control}$$

and

$$atom\%\ excess\ {}^{15}N_x = atom\%\ {}^{15}N_x - atom\%\ {}^{15}N_{control}$$

respectively, where $^{13}C_x$ and $^{15}N_x$ reflect the labelled sample atom% ^{13}C and ^{15}N, and $^{13}C_{control}$ and $^{15}N_{control}$ the mean of atom% ^{13}C and ^{15}N in control samples (i.e. natural isotopic abundance). In cases of negative atom% excess, values where set zero.

^{13}C, carbon and nitrogen in microbial biomass
Microbial biomass C and N was measured via chloroform fumigation extraction. 1.5 g of dried soil and litter samples were fumigated with chloroform under vacuum in the dark for 48 h. Fumigated and unfumigated samples were extracted with 15 ml of 0.5 M K_2SO_4, shaken for ½ h, filtered with Whatman® ashless filtration papers and subsequently stored at -20 °C until measurement (Brookes et al. 1985, Vance et al. 1987, Jenkinson et al. 2004). Aliquots of the K_2SO_4 extracts were used to measure total C and N in dissolved organic matter and microbial biomass on a TOC/TN analyser (TOC-VCPH Total organic carbon analyser, Shimadzu), and ^{13}C in the respective pools on a HPLC (Dionex) connected to a Finnigan LC-IsoLink Interface (Thermo Scientific). We did not include biomass conversion factors (k_{EC}, k_{EN}) into our calculations.
DOC and ^{13}C in DOC was derived from C and ^{13}C yield in unfumigated samples. Microbial biomass C (C_{mic}) was measured via CFE calculated as

$$C_{mic} = C_{fum} - C_{unfum}$$

and ^{13}C enrichment in microbial biomass as

$$\text{atom\% } {}^{13}C_{mic} = \frac{{}^{13}C_{mic}}{C_{mic}} \times 100$$

and consequently

$${}^{13}C_{mic} = \left(\frac{\text{atom\% } {}^{13}C_{fum}}{100} \times C_{fum}\right) - \left(\frac{\text{atom\% } {}^{13}C_{unfum}}{100} \times C_{unfum}\right)$$

where C_{mic} and $^{13}C_{mic}$ are C and ^{13}C in microbial biomass per g dry matter, respectively. 'fum' and 'unfum' indicate chloroform-fumigated and -unfumigated samples.

Phospholipid fatty acids (PLFAs)

Lipids were extracted based on method by Bligh and Dyer (1959), and fractionated as described in Frostegård et al. (1991). Samples were stored at -20 °C as suggested in Schnecker et al. (2012). PLFA analysis was done on rhizosphere soil, bulk soil and litter (n = 6). PLFA extraction and chromatographic analysis were carried out by Julia Wiesenbauer and Victoria Martin.

Lipid extraction and fractionation

0.5–2 g freeze–dried soil (and 0.2–0.5 g litter, respectively) was extracted with a single–phase mixture of chloroform, methanol and citrate buffer (1:2:0.8, v/v/v) for >6 h. After phase separation with chloroform and citrate buffer (1:1, v/v), the lower (organic) phase was collected and dried under a constant stream of N_2.

Lipids were fractionated on silica columns (Supelclean LC-Si 3 ml tubes, Supelco; pre–conditioned with chloroform) in consecutive order into neutral lipids (5 ml chloroform), glycolipids (20 ml acetone) and phospholipids (5 ml methanol, which was consequently collected). Phospholipids were converted to fatty acid methyl esters via alkaline methanolysis.

Dried samples were dissolved in 100 µl iso-octane and subsequently measured with gas chromatography–mass spectrometry (GC-MS) for quantification and gas chromatography–stable isotope ratio mass spectronomy (GC-IRMS) for determination of ^{13}C abundance. Nonadecanoic acid (PLFA 19:0) was added to samples before methylation and used as an internal standard for quantification, and bacterial and fungal fatty acid methyl esters (BAME CP mix, Supelco; 37 Component FAME mix, Supelco) as qualitative standards.

Gas chromatography–mass spectrometry

We analysed PLFA abundance on a gas chromatograph (Trace GC Ultra, Thermo Scientific) coupled to a mass spectrometer (ISQ, Thermo Scientific). Injection volume was 1 µl in splitless mode with a PTV injector. The GC temperature program was as follows: injection temperature at 70 °C holding for 1.5 min, then increase temperature at 30 °C min^{-1} to 150 °C holding for 1 min, and then increase temperature at 4 °C min^{-1} to 230 °C, which is held for 15 min. Injection, transfer line and ion source temperature were kept at 230, 240 and 270 °C, respectively.

We used a polar column (Agilent DB-23) with 0.25 mm i.d. and 0.25 mm film thickness, with helium as carrier gas at a constant flow of 1.5 ml min^{-1}.

Gas chromatography–isotope ratio mass spectrometry

^{13}C in PLFAs was measured on a Trace GC-Ultra coupled to a Delta V Advantage isotope ratio MS via a GC Isolink and Conflo IV (all instruments by Thermo Scientific). Injection volume was 1 µl in splitless mode with a SSL injector. The GC-temperature program, GC-column and carrier gas were the same as with the GC-MS measurement.

PLFA assignment

PLFAs were assigned to phylogenetic groups according to Schnecker et al. (2012) unless noted otherwise. i15:0, a15:0, i16:0, a16:0 (Lores et al. 2010, Kujur et al. 2014), i17:0 and a17:0 were used as indicator for Gram positive bacteria, 10Me16:0 and 10Me17:0 (Zelles 1999) for Actinobacteria, cy17:0 and cy19:0 for Gram negative bacteria, the sum of the above and 17:1ω7 (Kaiser et al. 2010) for bacteria in general, cis18:1ω9, trans18:1ω9 and 18:2ω6,9 for fungi in general, and 16:0 and 18:0 for general PLFAs.

Scanning electron microscopy and NanoSIMS

Sample preparation for NanoSIMS and scanning electron microscope (SEM) imaging was carried out by Marlies Dietrich. Sample hyphae were collected from the mesh barrier forming the inner boundary of the litter compartment. After air-drying, they were mounted on a Vectabond-coated silicon wafer suitable for both NanoSIMS and scanning electron microscope. In short, wafers were incubated in acetone for 5 min, then in a solution of acetone with a minimal amount of Vectabond reagent for 5 min, then washed in Milli-Q ultrapure water for 2 min, and subsequently let to dry at room temperature.

Following scanning electron microscopy, NanoSIMS analysis was carried out by Arno Schintlmeister (Large-Instrument Facility for Advanced Isotope Research, University of Vienna, Austria). For further information see Dietrich (2016).

NanoSIMS images were analysed with the FIJI package based on ImageJ, using the plugin OpenMIMS. Images were corrected for position inaccuracy via auto-tracking, for detection inaccuracy via dead time correction, and for quasi-simultaneous arrival via QSA-correction. The final image, obtained via stack accumulation, consisted of several images to cover the level of interest on the z-axis and to improve signal count.

Statistical analysis

R (R 3.3.2) was used for all statistical analyses, with package 'ggplot2' for plotting (Wickham, 2009) and package 'vegan' for multivariate analyses (Oksanen et al. 2016). Means or medians were considered to be significantly different from each other if $p < 0.05$. Since data transformations did not result in normal distribution or homogeneity of variance (which we account to the high variability in root biomass; see Table S1), non-parametric tests were used in all statistical analysis (Mann-Whitney U test for equal medians for two-sample comparisons, and Kruskal-Wallis rank sum test optionally followed by Dunn's post-hoc test of multiple comparisons with Bonferroni correction for multiple-sample comparisons). Outliers which were deleted for correspondence analysis were defined as those obser-

vations that lie outside 1.5 × the inter quartile range (i.e. the difference between 75th and 25th quartiles).

2.3 Results

Allocation of recently photoassimilated carbon

Analysis of ^{13}C in bulk and microbial pools shows rapid belowground allocation of recent photosynthates. 17-24 h after the start of the 6-hour ^{13}C-CO_2 exposure, photoassimilated ^{13}C was detected in root-inaccessible litter compartments (Fig. 2.3). While the majority of ^{13}C allocation was restricted to plant biomass, 11.74 % of total ^{13}C is allocated to soil and litter pools (Table 2.1). Moreover, plant-borne ^{13}C was incorporated into soil microbial biomass in both soil and litter (Fig. 2.4).

Photoassimilated carbon in fungal biomass

Since the only possibility for ^{13}C to reach the hyphae-only litter compartment is via ECM hyphae, isotopic enrichment in this compartment suggests transport via the mycorrhizal pathway. Significant ^{13}C enrichment was measured in litter (Fig. 2.3), accounting for 0.36 % of the total photoassimilated ^{13}C (Table 2.1). Furthermore, in both soil and litter pools, ^{13}C enrichment was observed in microbial biomass based on CFE (Fig. 2.4), as well as in fungal- and bacteria-specific PLFAs (Fig. 2.5, Table 2.2).

From the two fungal-specific PLFAs 18:1w9 and 18:2w6,9 most ^{13}C was incorporated into 18:2ω6,9 in rhizosphere soil, but equal isotopic enrichment was measured in litter. Interestingly, 18:1ω9 and 18:2ω6,9 differed in their relative distribution of ^{13}C incorporation between pools: isotopic enrichment stays relatively constant in 18:1ω9, with a slight decrease in bulk soil, whereas isotopic enrichment in 18:2ω6,9 decreases significantly in the litter compartment (Fig. 2.5).

NanoSIMS imaging provides qualitative evidence of ECM fungal ^{13}C allocation by enabling *in situ* visualisation of ^{13}C within mycorrhizal hyphae. Fig. 2.6 shows significant enrichment within a hypha obtained from the litter compartment, with highest region-of-interest measurements of up to 3.5 atom% excess ^{13}C. Apparent local accumulation of ^{13}C is due to the three dimensional structure of the sample.

Transfer of photoassimilated carbon to soil bacteria via mycorrhizal hyphae

Bacteria-specific PLFAs were enriched with ^{13}C in both soil and litter pools (Fig. 2.5). Although not all individual bacteria-specific PLFAs showed significant isotopic enrichment (not significantly enriched PLFAs were a16:0, 17:1ω7 and cy19:0), each bacterial group contained at least one significantly enriched PLFA biomarker in rhizosphere soil (except for PLFAs specific for general bacteria) and litter (Table 2.3). ^{13}C was preferentially allocated to PLFAs specific for Gram positive bacteria (excluding Actinobacteria), showing 4× and 2× higher atom% excess ^{13}C values than Gram negative bacteria in rhizosphere soil and litter, respectively. Suprisingly—and in contrast to the fungal biomarker 18:2w6,9—relative excess of ^{13}C in bacterial biomarkers was similarly high in the litter compartment as it was in the rhizosphere (Fig. 2.5). Together with the fact that dissolved organic carbon (DOC) was significantly enriched in ^{13}C in litter (Table 2.3), and isotopic enrichment in PLFAs tended to be lowest in bulk soil (Fig. 2.4), this suggests bacterial uptake of plant-borne C as ECM hyphal exudates presumable in the litter compartment.

This is qualitatively supported by NanoSIMS imaging of ^{13}C enrichment in accumulated exudation products and microbial cells on the surface of mycorrhizal hyphae obtained from the litter compartment, where up to 1.7 atom% access ^{13}C were detected (Fig. 2.7). Enriched structures outside of hyphae were confirmed as

cells by comparing morphologies in region of interests of the SEM with P-concentrations in the ^{31}P image (not shown; analysis performed by Dagmar Woebken, Stephanie Eichorst and Marlies Dietrich).

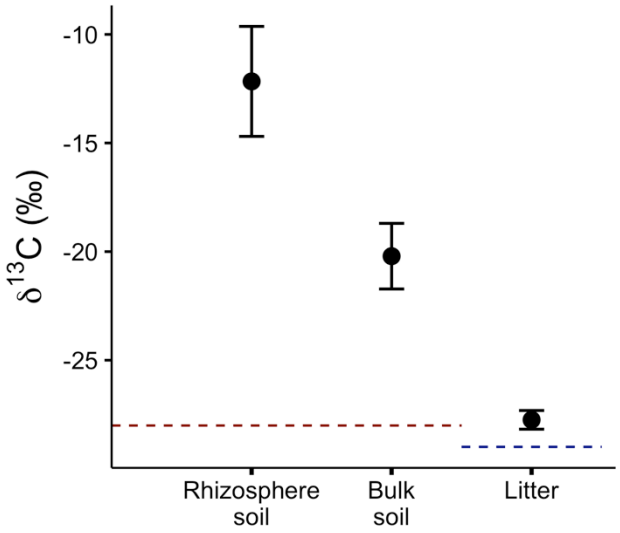

Figure 2.3 Belowground allocation of photosynthetically acquired ^{13}C in rhizosphere soil, bulk soil and litter pools, shown as $\delta^{13}C$. Dashed lines represent control values for soil (red; mean of rhizosphere and bulk soil), and litter (blue). Soil and litter pools were significntly different from control ($p < 0.05$). Error bars represent standard error; n = 7.

Table 2.1 Total C, ^{13}C, N and ^{15}N per box (in absolute values), percent of total ^{13}C and ^{15}N showing allocation of recent photosynthates and N from the litter compartment in bulk pools, and C/N ratio of bulk pools. ^{13}C and ^{15}N values are in excess, i.e. control was substracted. Standard errors are shown in brackets; n = 7.

Pool	**C (g)**	**^{13}C excess (mg)**	**% ^{13}C of total ^{13}C**	**N (g)**	**^{15}N excess (µg)**	**% ^{15}N of total ^{15}N**	**C/N**
Leaves	0.49 (0.05)	4.4 (0.64)	20.86 (2.74)	0.03 (0.00)	0.32 (0.14)	0.10 (0.05)	19.01 (0.49)
Stem	2.07 (0.19)	7.41 (0.48)	35.92 (2.21)	0.03 (0.00)	3.72 (1.73)	1.21 (0.58)	62.74 (4.09)
Roots	0.88 (0.14)	6.57 (0.74)	31.47 (3.04)	0.02 (0.00)	16.11 (4.66)	5.71 (1.99)	36.98 (1.37)
Plant biomass	3.44 (0.31)	18.37 (1.10)	88.26 (1.35)	0.08 (0.01)	20.15 (5.09)	7.02 (2.12)	-
Rhizosphere soil	6.70 (1.29)	0.96 (0.22)	4.35 (0.76)	0.53 (0.10)	13.22 (3.41)	4.25 (1.12)	12.46 (0.14)
Bulk soil	25.64 (1.88)	1.52 (0.34)	7.04 (1.25)	2.05 (0.16)	35.77 (13.48)	10.25 (3.07)	12.51 (0.06)
Litter	6.23 (0.15)	0.08 (0.03)	0.36 (0.13)	0.24 (0.00)	250.48 (18.46)	78.48 (3.56)	25.7 (0.39)
Soil + Litter	38.57 (1.72)	2.56 (0.43)	11.74 (1.35)	2.83 (0.13)	299.46 (26.07)	92.98 (2.12)	-
Total	42.01 (1.88)	20.93 (1.48)	100	2.91 (0.14)	319.62 (8.47)	100	-

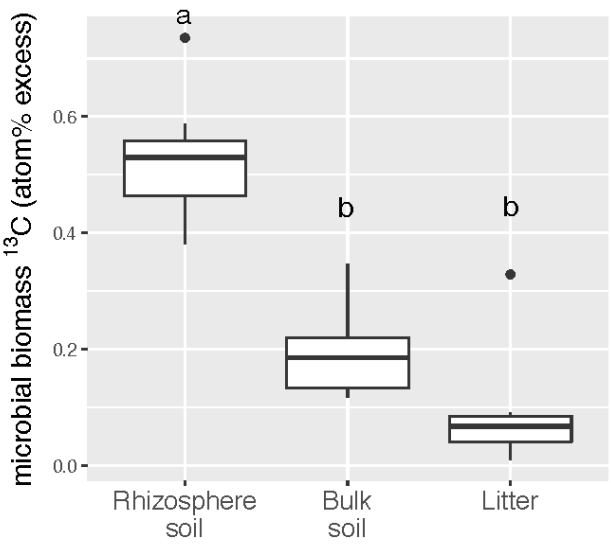

Figure 2.4 Belowground allocation of recent photosynthates to microbial biomass (measured by chloroform fumigation extraction) in root-accessible (soil) and hyphae-accessible pools (litter). Differences in pools were analyzed with Kruskal-Wallis rank sum test, followed by Dunn's post-hoc test of multiple comparisons with Bonferroni correction (adj. $p < 0.05$); $n = 8$.
Atom% ^{13}C values were compared against unlabelled controls via comparison via Mann-Whitney U test for equal medians. All pools were significantly enriched in ^{13}C compared to controls (not shown).

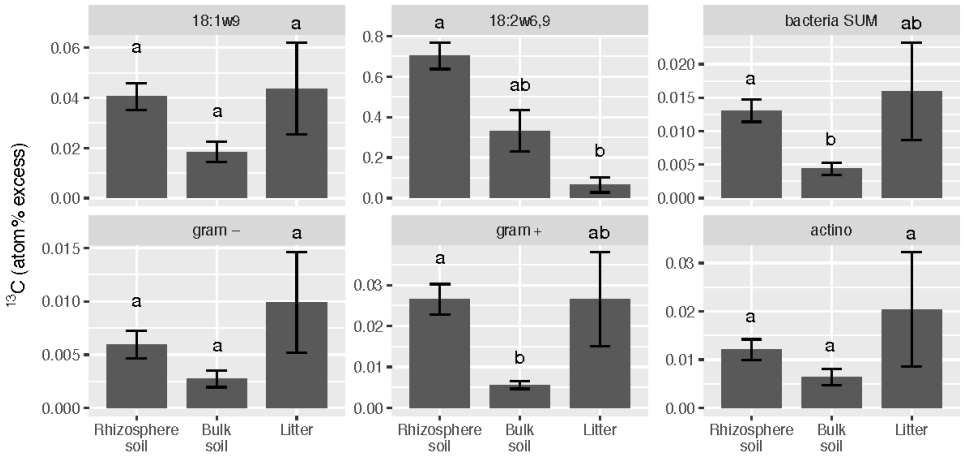

Figure 2.5 Enrichment of ^{13}C in fungal-specific (18:1ω9 and 18:2ω6,9) and bacteria-specific PLFAs in soil and litter compartments. Litter compartment enrichment indicates allocation of ^{13}C via ECM hyphae to root-distant bacteria.
Differences in pools were analyzed with Kruskal-Wallis rank sum test. Significant tests ($p < 0.05$) were followed by Dunn's post-hoc test of multiple comparisons with Bonferroni correction (adj. $p < 0.05$); $n = 6$. Error bars represent standard error.

Table 2.2 Atom% excess ^{13}C in PLFAs. Values are means, standard error is shown in brackets. *, significant difference to control ($p > 0.05$) in atom% ^{13}C, comparison via Mann-Whitney U test for equal medians; n = 6. †, control values were not detected, thus the mean of control values of other PLFAs with the same specificity were used as control; n.d., not detected.

		Rhizosphere soil		**Bulk soil**		**Litter**	
Phylogenetic specificity	**PLFA**	**-N**	**+N**	**-N**	**+N**	**-N**	**+N**
Fungi	cis18:1ω9	0.05* (0.01)	0.036* (0.007)	0.022* (0.006)	0.017* (0.004)	0.073* (0.038)	0.025* (0.008)
	trans18:1ω9	0.029* (0.003)	0.022* (0.004)	0.012* (0.003)	0.012* (0.003)	0.039*† (0.025)	0.008† (0.005)
	18:2ω6,9	0.656* (0.089)	0.75* (0.071)	0.236 (0.052)	0.428 (0.182)	0.074* (0.055)	0.056* (0.026)
Actinobacteria	10Me16:0	0.019* (0.004)	0.015* (0.004)	0.008 (0.002)	0.009 (0.003)	0.035* (0.024)	0.01* (0.003)
	10Me17:0	0.004* (0.001)	0.002* (0.001)	0.002 (0.001)	0.003 (0.001)	0.018* (0.012)	0.011* (0.003)
Bacteria general	17:0	0.022 (0.005)	0.016 (0.005)	0.011 (0.003)	0.01 (0.003)	0.02* (0.007)	0.013* (0.008)
	17:1ω7	0.001 (0.001)	n.d.	0.002 (0.001)	0.002 (0.001)	0.003 (0.002)	n.d.
General	16:0	0.164* (0.024)	0.194* (0.038)	0.058 (0.014)	0.09 (0.038)	0.043* (0.016)	0.023* (0.007)
	18:0	0.087* (0.025)	0.104* (0.019)	0.03 (0.01)	0.052 (0.023)	0.027* (0.01)	0.018* (0.007)
Gram negative bacteria	cy17:0	0.004* (0.001)	0.004 (0.001)	0.002 (0.001)	0.004* (0.001)	0.015* (0.008)	0.009* (0.003)
	cy19:0	0.007 (0.003)	0.006 (0.002)	0.003 (0.001)	0.002 (0.001)	0.012 (0.007)	0.007 (0.002)
Gram positive bacteria	a15:0	0.003 (0.001)	0.003 (0.001)	0.003 (0.001)	0.003* (0.001)	0.028* (0.017)	n.d.
	a16:0	0.718† (0.227)	0.179 (0.179)	0.001 (0.001)	0.001 (0)	0.179 (0.179)	n.d.
	a17:0	0.007* (0.002)	0.005 (0.002)	0.005 (0.001)	0.005* (0.001)	0.025* (0.014)	0.021* (0.009)
	i15:0	0.008 (0.002)	0.007 (0.002)	0.006 (0.002)	0.005* (0.001)	0.017* (0.011)	n.d.
	i16:0	0.012* (0.002)	0.018* (0.004)	0.006 (0.002)	0.008* (0.001)	0.029* (0.015)	0.021* (0.011)
	i17:0	0.013* (0.002)	0.01* (0.002)	0.007* (0.002)	0.007* (0.001)	0.026* (0.016)	0.021* (0.008)

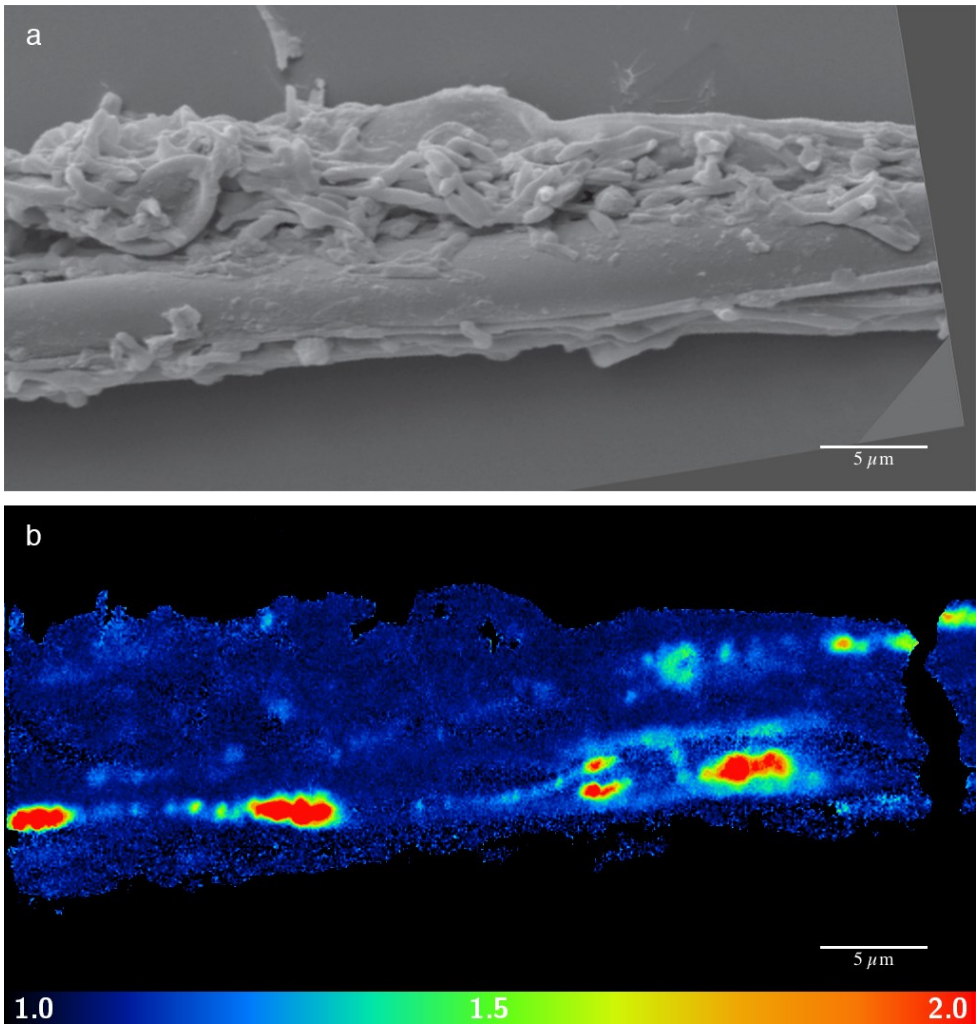

Figure 2.6 NanoSIMS imaging of fungal hyphae extracted from a litter compartment. Panels depict (a) an SEM image of hyphae, and (b) the corresponding carbon isotope composition image, at an erosion depth below hyphal surface, showing ^{13}C enrichment inside root-distant hyphae. Isotopic concentrations are given in atom% ($^{13}C/(^{12}C+^{13}C)$). The lower end of the scale is set at natural abundance (1.08 atom% ^{13}C), maximum values are 2.0–3.6 atom% ^{13}C. Signal intensity threshold is set at 150 counts pixel^{-1}. Images kindly provided by Marlies Dietrich, Arno Schintlmeister, Stephanie Eichorst and Dagmar Woebken and further processed by myself.

Table 2. 3 ^{13}C, C and N in dissolved organic matter and microbial biomass. No significant differences were detected between +N/–N compartments (comparison via Mann-Whitney U test for equal medians; n = 7), except for DOC in bulk soil. However, this seems to be an artefact, and was also found in controls where no N was added.
C_{mic}, N_{mic}, DOC and TDN are means in µg g^{-1} dry weight, and $^{13}C_{mic}$ and ^{13}C in DOC are atom% excess. C_{mic}, C in microbial biomass; $^{13}C_{mic}$, ^{13}C in microbial biomass; N_{mic}, N in microbial biomass; DOC, dissolved organic C; TDN, total dissolved N; C/N_{mic}, C/N ratio in microbial biomass. Standard error is shown in brackets. *, significant difference to control ($p > 0.05$) in atom% ^{13}C, comparison via Mann-Whitney U test for equal medians; n = 8.

Pool		**C_{mic}**	**N_{mic}**	**DOC**	**TDN**	**$^{13}C_{mic}$**	**^{13}C in DOC**	**C/N_{mic}**
Rhizosphere soil	–N	610.28	142.12	154.68	27.62	0.47*	0.17*	4.31
		(15.95)	(4.69)	(6.27)	(3.16)	(0.05)	(0.06)	(0.10)
	+N	665.6	141.96	139.26	29.98	0.61*	0.15*	4.70
		(24.63)	(5.94)	(3.79)	(7.4)	(0.06)	(0.06)	(0.12)
Bulk soil	–N	655.13	126.36	145.35	25.97	0.20*	0.03*	5.26
		(30.75)	(6.05)	(25.47)	(3.3)	(0.02)	(0.01)	(0.34)
	+N	620.07	132.21	179.85	36.2	0.18*	0.03*	4.77
		(18.81)	(7.75)	(11.92)	(7.86)	(0.04)	(0.01)	(0.24)
Litter	–N	1725.91	286.29	2867.67	811.58	0.10*	0.01*	6.22
		(137.64)	(33.68)	(112.47)	(45.18)	(0.05)	(0.00)	(0.44)
	+N	1822.26	259.31	3004.41	857.49	0.09*	0.01*	7.53
		(206.47)	(39.96)	(95.22)	(110.57)	(0.03)	(0.00)	(0.68)

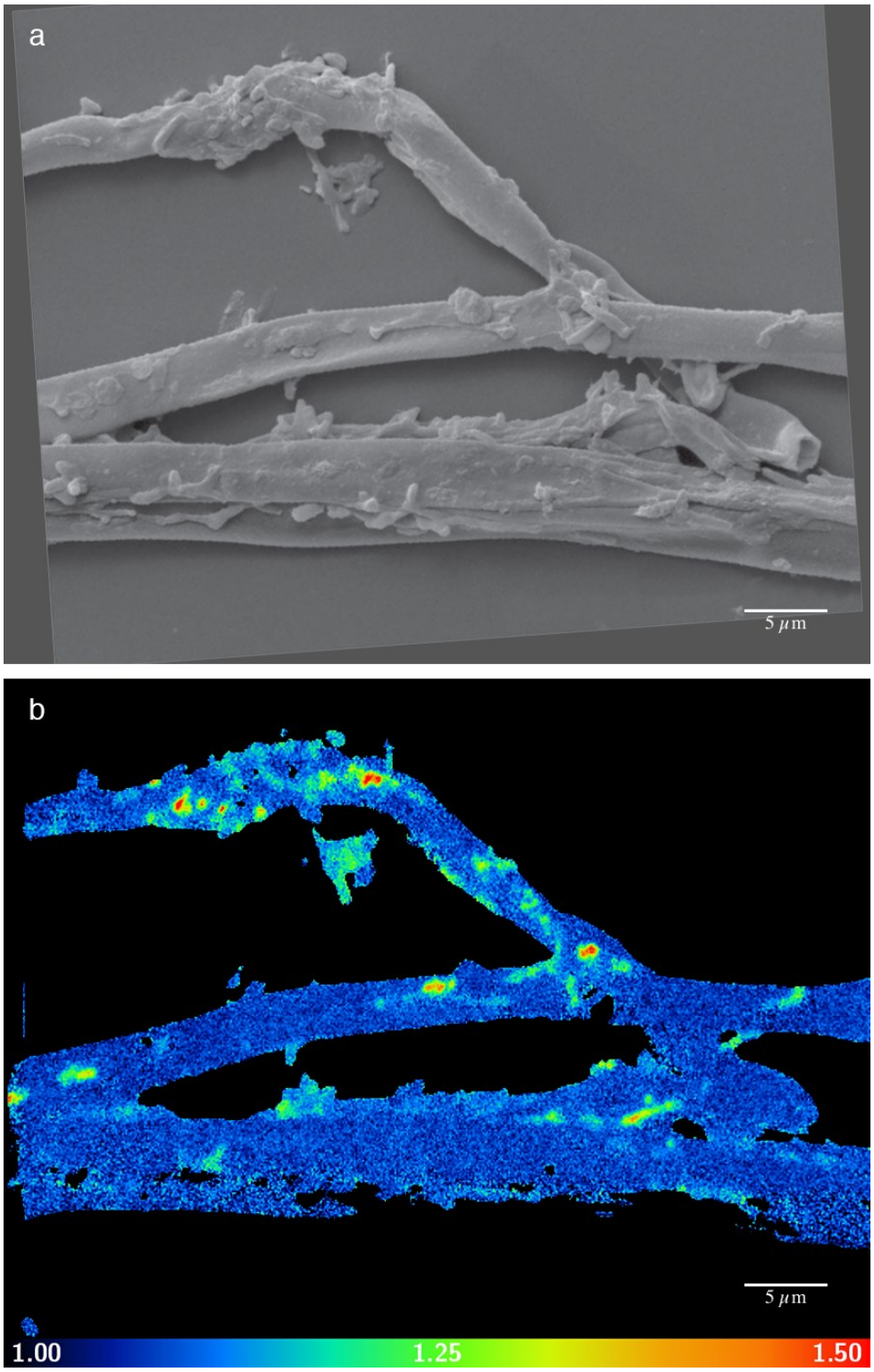

Figure 2.7 NanoSIMS imaging of cells attached to fungal hyphae extracted from a litter compartment. Panels depict (a) an SEM image, and (b) the corresponding carbon isotope composition image, showing ^{13}C enrichment in bacterial cells on the surface of root-distant hyphae. Isotopic concentrations are given in atom% ($^{13}C/(^{12}C+^{13}C)$). The lower end of the scale is set at natural abundance (1.08 atom% ^{13}C), maximum values are 1.50–1.56 atom% ^{13}C. Signal intensity threshold is set at 50 counts pixel^{-1}. Images kindly provided by Marlies Dietrich, Arno Schintlmeister, Stephanie Eichorst and Dagmar Woebken and further processed by myself..

Effect of nitrogen addition on belowground carbon flux and soil bacteria

Microbial reaction to nitrogen addition

PLFA analysis shows a significant decline in biomass of several microbial groups with N addition (Fig. 2.8, Table 2.4). The adverse effect of N was particularly pronounced in Actinobacteria and other Gram+ bacteria, while Gram- bacteria showed no response. Surprisingly, the adverse effect on Gram positive bacteria was not restricted to the litter compartment where N addition was applied, but also took place in untreated soil pools adjacent to the N-amended litter compartments (Fig. 2.8). In line with this, correspondence analysis of C in PLFAs shows that microbial communities are distinctly different not only between litter and soil, but also with N addition (Fig. 2.9).

In contrast to the PLFAs, there was no effect of N addition on C in microbial biomass based on CFE (Table 2.3). A reason for the divergence of precision in measuring microbial biomass between CFE and PLFA measurements may be observational differences in their methodologies: Phospholipids are restricted to cell membranes, and as such correlate to cell surface areas, while CFE lyses cells and correlates to cell volumes. If hyphae restrict C exudation, affected soil bacterial communities might switch from r-strategists with small cells to K-strategists with larger cells. Thus, the combined surface area of bacterial cells would decrease, while the total volume stays the same, giving lower yields of PLFAs but the same with CFE.

Difference in carbon allocation to soil microbes with nitrogen addition

There was no significant effect of N addition on ^{13}C in microbial biomass based on CFE (Table 2.3). Based on PLFA analysis, however, N addition to the litter compartment significantly decreased ^{13}C allocation to Actinobacteria and other Gram positive bacteria, on a per g soil or litter basis (Fig. 2.10, Table 2.5). This was caused by loss of biomass of the respective groups combined with a constant or decreased relative enrichment of ^{13}C. Relative isotopic enrichment in PLFAs specific for Gram positive bacteria and Actinobacteria, and the fungal-specific PLFA 18:1ω9, tended to decrease in litter after the addition of N (Fig. 2.11). Moreover, isotopic enrichment in Gram positive bacteria-specific PLFAs also seemed to decrease in rhizosphere soil (Fig. 2.11). The overall decrease of ^{13}C enrichment in bacteria-specific PLFAs with N-addition is strongly influenced by the decrease of ^{13}C in Gram-positive bacteria.

This effect is also illustrated by the multivariate correspondence analysis of atom% excess ^{13}C in PLFAs, where uptake of recent photosynthates by soil microbial groups differs not only between litter and soil pools, but also between N-treatment compartments in the litter pool (Fig. 2.12).

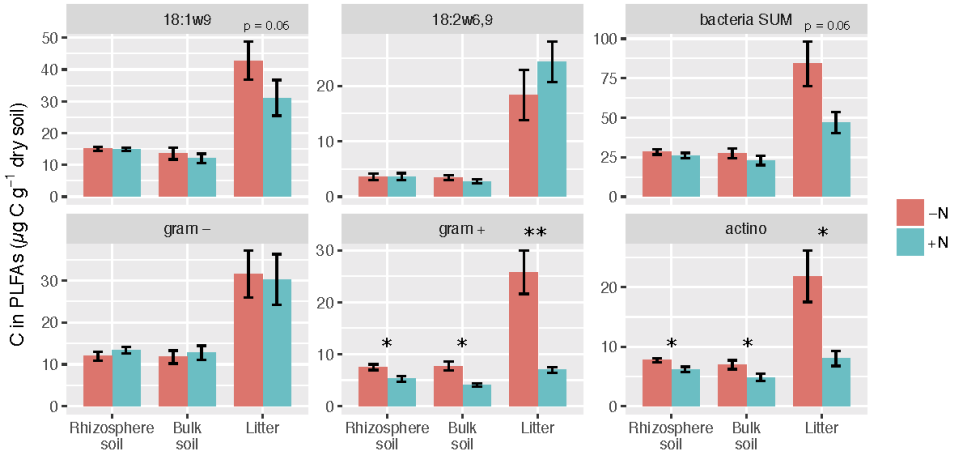

Figure 2.8 C in PLFAs used as proxy for biomass of fungi and bacteria. Biomass of Actinobacteria and Gram positive bacteria (excluding Actinobacteria) declined significantly in both litter and soil compartments with N addition (i.e. to the litter compartment). No effect was found for Gram negative bacteria or fungi.
Differences in pools of the untreated side (-N) were analyzed with Kruskal-Wallis rank sum test. Significant tests ($p < 0.05$) were followed by Dunn's post-hoc test of multiple comparisons with Bonferroni correction (adj. $p < 0.05$). Rhizosphere soil and litter, as well as bulk soil and litter, were significantly different in all groups except for 18:2ω6,9 and Actinobacteria (not shown).

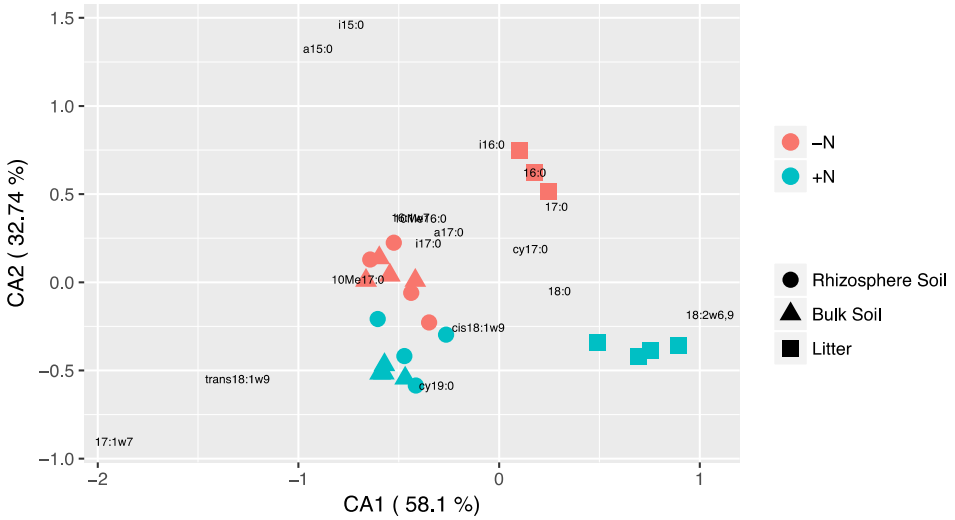

Figure 2.9 Correspondence analysis of C in PLFAs (µg C g^{-1} dry weight). Separation of the litter compartment from soil pools indicates a distinctly different microbial community. N addition affects not only community structure in hyphae-only litter compartments, but also in soil compartments.
PLFA data was deleted from certain pools in boxes when classified as outliers. Deleted values: box 8 –N (all pools); box 14 +N (Litter), –N (Litter); box 16 +N (bulk and rhizosphere soil); box 29 (all pools)

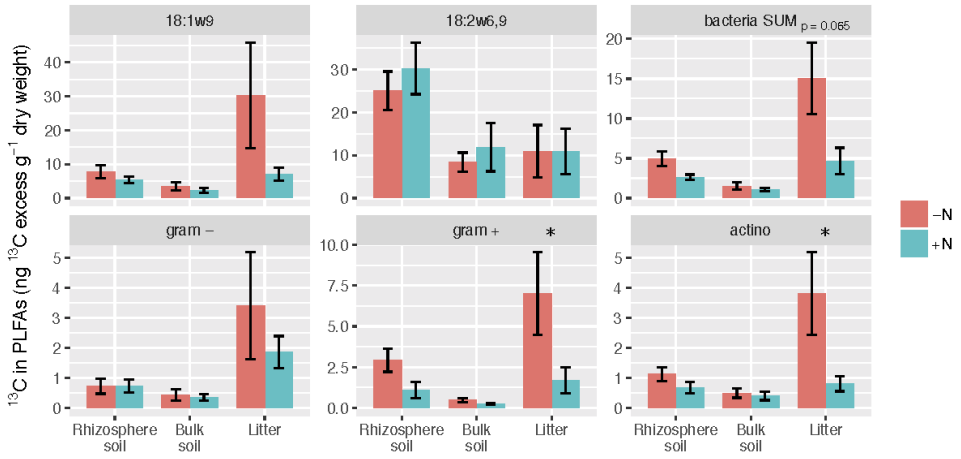

Figure 2.10 Enrichment of photosynthetically assimilated ^{13}C in PLFAs per g dry weight. Litter compartment enrichment of bacteria-specific PLFAs indicates allocation of ^{13}C via EM hyphae to root-distant bacteria. Asterisks indicate significant difference between N treatment (* $p < 0.05$; ** $p < 0.01$), comparison via Mann-Whitney U test for equal medians, $n = 6$. Error bars represent standard error.

Differences in pools of the untreated side (-N) were analyzed with Kruskal-Wallis rank sum test. Significant tests ($p < 0.05$) were followed by Dunn's post-hoc test of multiple comparisons with Bonferroni correction (adj. $p < 0.05$). Bulk soil and litter were significantly different in all groups except for 18:2ω6,9 and Gram negative bacteria (not shown).

Table 2.4 C in PLFAs, as µg C g^{-1} dry soil. Values are means, standard error is shown in brackets. n = 6; n.d., not detected.

		Rhizosphere soil		Bulk soil		Litter	
Phylogenetic specificity	**PLFA**	**-N**	**+N**	**-N**	**+N**	**-N**	**+N**
Fungi	cis18:1ω9	12.9 (0.55)	12.37 (0.39)	11.75 (1.54)	10.26 (1.08)	34.96 (5.19)	28.99 (4.55)
	trans18:1ω9	2.16 (0.23)	2.5 (0.19)	1.85 (0.37)	1.81 (0.36)	7.84 (3.82)	2.1 (1.18)
	18:2ω6,9	3.61 (0.57)	3.63 (0.62)	3.48 (0.45)	2.76 (0.34)	18.33 (4.5)	24.35 (3.67)
Actinobacteria	10Me16:0	5.13 (0.19)	3.94 (0.3)	4.68 (0.48)	3.14 (0.33)	15.76 (2.76)	5.51 (0.73)
	10Me17:0	2.61 (0.15)	2.25 (0.15)	2.27 (0.28)	1.69 (0.26)	6.06 (1.58)	2.52 (0.65)
Bacteria general	17:0	0.82 (0.12)	0.76 (0.05)	0.76 (0.05)	0.64 (0.14)	4.5 (0.76)	1.8 (0.48)
	17:1ω7	0.43 (0.03)	0.41 (0.01)	0.4 (0.09)	0.58 (0.32)	0.6 (0.39)	n.d.
General/unspecific	16:0	5.98 (0.23)	4.6 (0.23)	5.7 (0.16)	3.62 (0.06)	28.51 (2.73)	11.64 (0.85)
	18:0	5.56 (1.12)	6.49 (0.27)	5.1 (1.07)	5.68 (0.4)	26.76 (1.81)	21.69 (1.13)
Gram negative bacteria	cy17:0	2.26 (0.1)	2.05 (0.1)	2.28 (0.19)	1.81 (0.19)	9.24 (0.75)	5.98 (0.29)
	cy19:0	9.68 (0.93)	11.31 (0.7)	9.46 (1.38)	10.94 (1.52)	22.32 (5)	24.28 (5.91)
Gram positive bacteria	a15:0	0.93 (0.15)	0.42 (0.14)	1.17 (0.22)	0.25 (0.06)	2.89 (0.69)	n.d.
	a16:0	0.19 (0.06)	0.05 (0.05)	0.25 (0.06)	0.12 (0.04)	0.23 (0.23)	n.d.
	a17:0	2.04 (0.09)	1.7 (0.12)	1.83 (0.16)	1.33 (0.15)	6.59 (0.98)	2.89 (0.21)
	i15:0	0.97 (0.15)	0.46 (0.15)	1.16 (0.17)	0.3 (0.06)	3.73 (0.68)	n.d.
	i16:0	1.29 (0.09)	0.93 (0.07)	1.39 (0.12)	0.72 (0.03)	6.02 (0.66)	1.78 (0.39)
	i17:0	2.07 (0.09)	1.71 (0.12)	1.87 (0.17)	1.37 (0.13)	6.3 (1.24)	2.3 (0.31)

Table 2.5 ^{13}C in PLFAs, as ng ^{13}C g^{-1} dry soil. Values are means, standard error is shown in brackets. n = 6; n.d., not detected.

		Rhizosphere soil		Bulk soil		Litter	
Phylogenetic specificity	**PLFA**	**-N**	**+N**	**-N**	**+N**	**-N**	**+N**
Fungi	cis18:1ω9	7.12 (1.81)	4.81 (0.92)	3.24 (1.11)	2.03 (0.59)	22.35 (8.95)	6.75 (1.84)
	trans18:1ω9	0.68 (0.1)	0.61 (0.14)	0.28 (0.06)	0.27 (0.11)	7.97 (7.24)	0.26 (0.13)
	18:2ω6,9	25.02 (4.49)	30.25 (6.03)	8.41 (2.21)	11.86 (5.63)	10.95 (6.09)	10.89 (5.31)
Actinobacteria	10Me16:0	1.03 (0.21)	0.62 (0.17)	0.44 (0.14)	0.36 (0.14)	3.22 (1.18)	0.57 (0.2)
	10Me17:0	0.09 (0.02)	0.06 (0.02)	0.05 (0.02)	0.03 (0)	0.58 (0.2)	0.23 (0.05)
Bacteria general	17:0	0.19 (0.04)	0.13 (0.04)	0.09 (0.02)	0.07 (0.02)	0.8 (0.23)	0.31 (0.17)
	17:1ω7	0.01 (0.01)	n.d.	0.01 (0.01)	0.01 (0)	0.04 (0.04)	n.d.
General	16:0	10.4 (1.34)	9.57 (2.03)	3.64 (0.88)	3.49 (1.45)	11.46 (3.67)	2.91 (1.08)
	18:0	6.34 (1.86)	7.1 (1.18)	2.13 (0.74)	3.57 (1.76)	7.23 (2.17)	4.17 (1.73)
Gram negative bacteria	cy17:0	0.1 (0.02)	0.09 (0.02)	0.06 (0.02)	0.09 (0.04)	1.19 (0.53)	0.59 (0.21)
	cy19:0	0.62 (0.24)	0.65 (0.21)	0.37 (0.16)	0.26 (0.11)	2.21 (1.25)	1.27 (0.4)
Gram positive bacteria	a15:0	0.03 (0.01)	0.02 (0.01)	0.04 (0.01)	0.01 (0)	0.4 (0.08)	n.d.
	a16:0	2.21 (0.72)	0.57 (0.57)	n.d.	n.d.	2.68 (2.68)	n.d.
	a17:0	0.15 (0.02)	0.1 (0.03)	0.1 (0.03)	0.07 (0.01)	1.19 (0.38)	0.66 (0.31)
	i15:0	0.08 (0.02)	0.04 (0.02)	0.09 (0.02)	0.02 (0.01)	0.34 (0.09)	n.d.
	i16:0	0.16 (0.02)	0.18 (0.04)	0.09 (0.03)	0.07 (0.01)	1.43 (0.43)	0.57 (0.34)
	i17:0	0.28 (0.03)	0.18 (0.05)	0.16 (0.04)	0.1 (0.01)	0.96 (0.27)	0.47 (0.14)

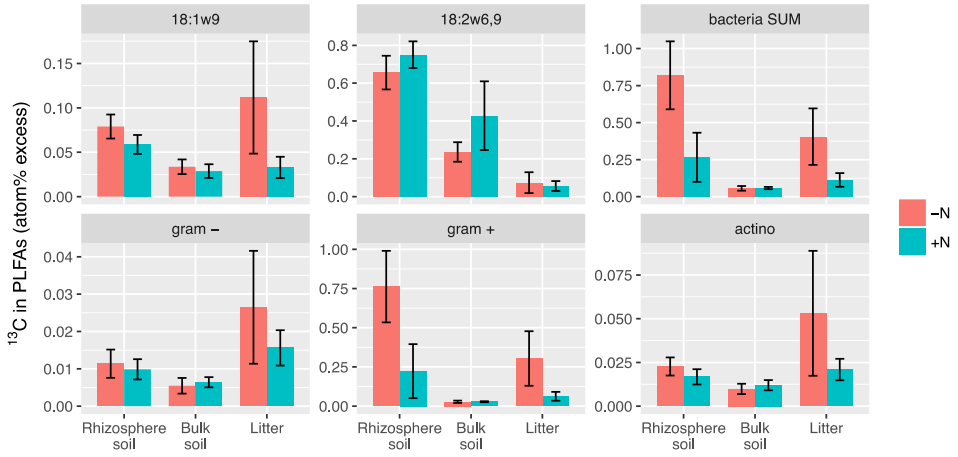

Figure 2.11 Enrichment of photosynthetically assimilated ^{13}C in PLFAs in atom% excess ^{13}C. Litter compartment enrichment of bacteria-specific PLFAs indicates allocation of ^{13}C via EM hyphae to root-distant bacteria. No significant differences could be detected by comparison via Mann-Whitney U test for equal medians. Error bars represent standard error; n = 6.

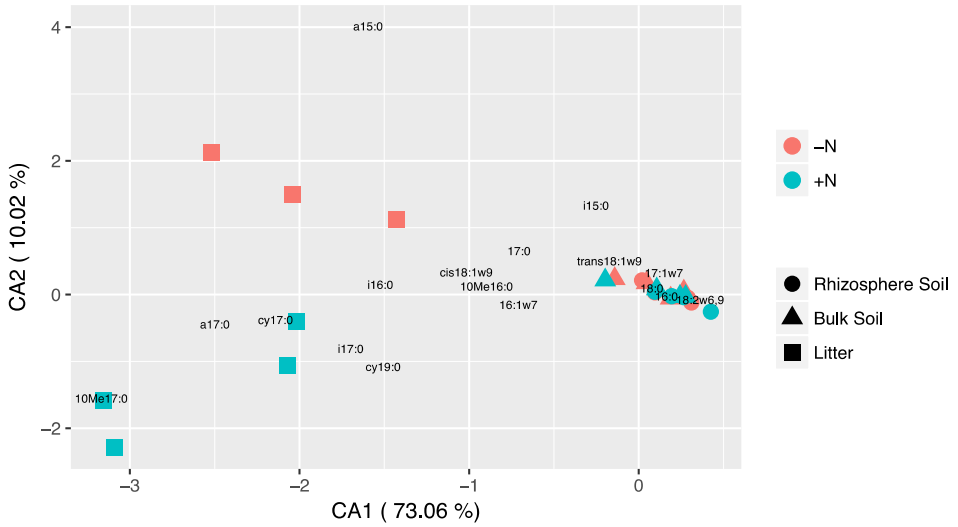

Figure 2.12 Correspondence analysis of atom% excess ^{13}C in PLFAs. It shows that ^{13}C was allocated to different microbes in soil and litter pools, respectively. Also, with N addition ^{13}C was allocated to a different microbial community in the litter compartment. We argue that this reflects biomass change and not preferential ^{13}C allocation to specific taxonomic groups (see Fig. 2.8 and 2.9 for comparison).

PLFA data was deleted from certain pools in boxes when classified as outliers. Deleted values: box 8 –N (all pools); box 14 +N (Litter), –N (Litter); box 16 +N (bulk and rhizosphere soil); box 29 (all pools)

Bidirectionality and reciprocity of carbon and nitrogen exchange

We detected enrichment of ^{15}N in soil and plant biomass (Fig. 2.13). In fact, all pools were significantly enriched compared to controls, except for plant leaves. Of the total ^{15}N added, 21.52 % (± 3.56 standard error) were transferred out of the litter compartments, and 7.02 % (± 2.12 standard error) were taken up into plant biomass. Taken together our results indicate—as expected—bidirectional transport of ^{13}C photo-oxidized in plant leaves (Fig. 2.3) and ^{15}N from the litter compartment (Fig. 2.13) via ECM hyphae in our experiment.

However, no effect of N addition on ^{13}C allocation was found at the bulk root scale by statistical comparison of atom% ^{13}C excess in bulk roots between N-amended and untreated side (Fig. 2.14). Nonetheless, there was a significant correlation between isotope atom% excess of ^{15}N and ^{13}C when separating bulk roots of the N-amended side of each box into smaller root pieces based on root system architecture ($R^2 = 0.161$, $p = 0.006$; Fig. 2.15).

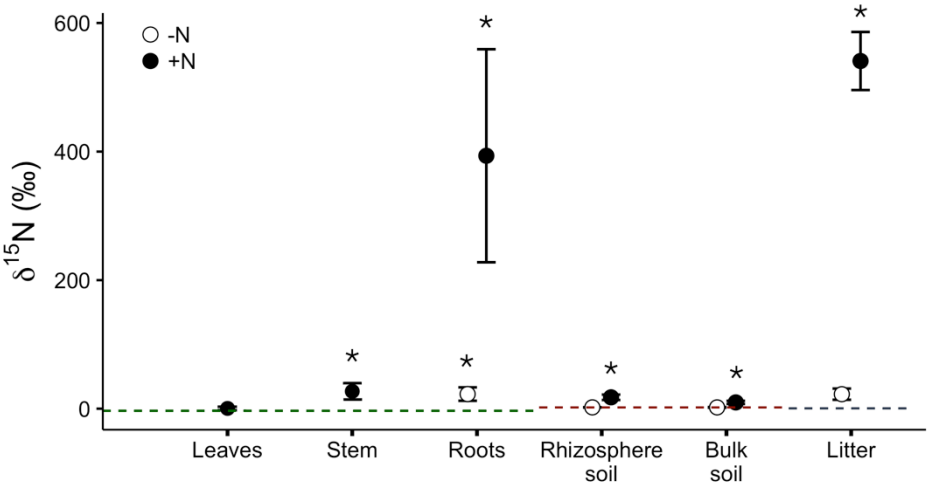

Figure 2.13 Belowground allocation of ^{15}N added in the hyphae-accessible litter compartment in bulk pools, shown as $\delta^{15}N$. These results indicate allocation of ^{15}N via EM hyphae from the litter compartment to plant roots. Significant ^{15}N-enrichment of plant tissues was even detected in roots of the unlabelled compartment, and the stem of the plant.

Dashed lines represent control values for plant biomass (green; mean between leaves, stem and roots), soil (red; mean between rhizosphere and bulk soil), and litter (blue). Asterisks indicate significant difference from control (* $p < 0.05$; comparison via Mann-Whitney U test for equal medians). Error bars represent standard error; $n = 7$.

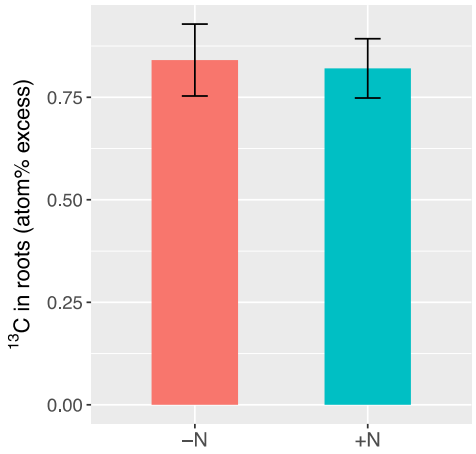

Figure 2.14 Enrichment of ^{13}C in bulk roots of N addition and untreated side. No difference between treatments is apparent. Comparison via Mann-Whitney U test for equal medians; n = 7.

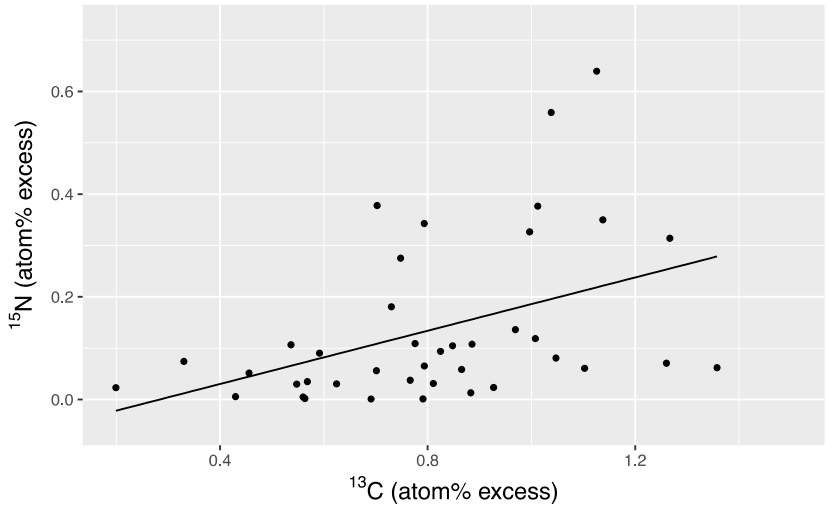

Figure 2.15 Isotopic enrichment (^{13}C and ^{15}N) in individual root pieces of the ^{15}N-amended side gives a weak fit for linear regression. Each bulk root was separated into smaller root pieces based on root system architecture (3-4 pieces root^{-1}). Points reflect isotope atom% excess in individual root pieces. *Linear regression*: adj. $R^2 = 0.161$, $p = 0.006$.

While we did not detect differences in bulk roots with N addition in ^{13}C allocation (Fig. 2.14), this weak linear fit of isotopic enrichments at a lower level of root system architecture hints reciprocal exchange of C and N at lower special scales. It is noteworthy that ^{13}C was always allocated to roots (i.e. atom% excess ^{13}C are never zero), whereas ^{15}N allocation seemed to partially depend on ^{13}C allocation (i.e. low ^{13}C allocation equals low, often zero, ^{15}N allocation; but high ^{15}N allocation was only observed when ^{15}N allocation was also high).

2.4 Discussion

It has been hypothesized that mycorrhizal fungi release C, which they receive from their host plant, in root distant areas. If so, this labile C input would stimulate activity of free-living saprotrophs in the hyphosphere (Hodge et al. 2010, Jansa et al. 2013), liberating nutrients from increased decomposition of organic matter, which could then be acquired and transferred to the plant host by ECM fungi (Finlay et al. 1988, 1989, Pena et a. 2013). It has further been hypothesized that plants may be able to adjust their C flow according to the amount of N offered by the fungus (Kiers & van der Heijden 2006, Hammerstein & Noë 2016).
We found that plant-borne C is rapidly allocated to root-distant soil bacteria via ECM hyphae, supporting the hypothesis of priming in an ECM hyphosphere. Plants could not discriminate against fungi which provided less N to one half of the root system. However, we detect hints of reciprocal control at a lower scale of the root system architecture.

Rapid belowground allocation of recently photoassimilated carbon to soil microbes

Our results demonstrate rapid belowground allocation of recent photosynthates. 17-24 h after the start of ^{13}C-CO_2 pulse labelling, 11.7 % of the total assimilated ^{13}C was allocated belowground outside of plant roots. In this study, belowground C fluxes represent conservative estimates, as belowground respiration was not accounted for, which can account for a large fraction of plant gross primary production (Kucey & Paul 1981, Rygiewicz & Andersen 1994). Still, compared to other studies, belowground C allocation measured in this study is low. For instance, Hobbie (2006) found C allocation in pure culture studies accounted for 27-68 % of plant net primary production. However, our comparably short ^{13}C-CO_2 incubation and accumulation time (6 and 11-18 h respectively) may distort an estimation of overall C flow in this experimental system.

Ectomycorrhizal fungi transfer recent photosynthates to root-distant areas

Our results indicate rapid transport of recent photosynthates to root-distant areas via ECM hyphae. This is supported by the finding that a significant fraction (3.0 %) of the total ^{13}C allocated outside plant roots were recovered in the litter compartment. Since root growth into the litter compartment was restricted by a mesh only hyphae could penetrate, and the double-layer mesh design with an air-filled space inbetween restricted diffusion between the two compartments, transport of plant-derived C is theoretically limited to mycorrhizal hyphae. Furthermore, we observed significant isotopic enrichment in microbial biomass, as well as in fungal-specific PLFAs 18:1ω9 and 18:2ω6,9, in soil and litter. Additionally, NanoSIMS imaging provides qualitative evidence of significant ^{13}C enrichment inside fungal hyphae obtained from the litter compartment.
Both PLFAs 18:1ω9 and 18:2ω6,9 are frequently used biomarkers for fungal biomass in soil, and 18:2ω6,9 has been observed to show good correlations with mycorrhizal colonisation on roots in an ECM dominated forest (Kaiser et al. 2010). However, both biomarkers are also found in plant tissues which may introduce a bias due to small amounts of fine roots remaining in sieved soil samples (Frostegård et al. 2011). Although contribution of root-borne PLFAs 18:1ω9 and 18:2ω6,9 to soil has been demonstrated to be negligible (Kaiser et al. 2010), highly labelled plant material may contribute significantly to the labelled PLFA pool,

especially in rhizosphere soil. A potential solution would be the additional measurement of ergosterol, a biomarker exclusively specific to fungi. However, so far there is no established methodology for quantifying ^{13}C in ergosterol available. Nevertheless, ^{13}C enrichment in fungal-specific PLFAs in the litter compartment are assumed to be of sole ECM fungal origin, as root access was restricted.
Interestingly, the two fungal specific PLFAs differed in their relative distribution and ^{13}C incorporation: 18:1ω9 took up less ^{13}C, but was more abundant than 18:1ω6,9 (Fig. 2.5, untreated side in Fig. 2.8). We therefore suggest that 18:1ω9 to be a more general marker for fungi, including free living saprotrophs, while 18:2ω6,9 is more specific for ECM fungi. Furthermore, 18:1ω9 decreases in abundance in litter with N addition, showing the same reaction as bacteria (Fig. 2.8), providing additional support for this hypothesis.

Soil bacteria receive recent photosynthates from ectomycorrhizal fungi
Providing free-living saprotrophs with energetically advantageous low-molecular weight C compounds is a potentially viable strategy for ECM fungi to accelerate decomposition of recalcitrant organic matter. Significant isotopic enrichment of ^{13}C-DOC and ^{13}C-PLFAs specific for bacterial groups in the litter compartment suggests bacterial uptake of ECM fungal exudates derived from recent photosynthates. Most plant-derived ^{13}C was transferred to Gram positive bacteria. This stands in direct contrast to previous studies, which detected Gram negative bacteria to preferentially utilize plant-derived compounds. Such was observed in both AM (Kramer & Gleixner 2006, 2008) and ECM systems (Esperschütz et al. 2009, Pickles et al. 2016).
Since priming effects probably involve both fungal and bacterial saprotrophic organisms, the possibility that ^{13}C exuded by ECM fungi was acquired by saprotrophic fungi cannot be excluded (Fernandez & Kennedy 2015). This is in fact supported by reduction in biomass and absolute ^{13}C incorporation with N addition of the fungal PLFA 18:1ω9 (Fig. 2.8, Fig. 2.10; as discussed below), which we think to be a general marker for fungi (see above).
In absolute values, bacteria incorporated most ^{13}C in the litter compartment (untreated side in Fig. 2.10). This points to increased hyphal C exudation in the litter compartment compared to the soil compartment. When long-distance fungal hyphae reach nutrient-rich substrates, they increase branching and growth (Agerer 2001, 2006), subsequently increasing the mycelial surface area and number of root tips (Katz et al. 1972). Hyphal exudation has been observed to concentrate on ECM hyphal tips (Unestam & Sun 1995, Sun et al. 1999), and to increase bacterial activity by providing water, C and N (Worrich et al. 2017). Indeed, it could be a viable strategy for ECM fungi to achieve higher priming of free-living saprotrophs in substrates where N is scarce. Although litter showed higher C/N ratios compared to soil, total dissolved N was disproportionately higher in litter. At the same time, dissolved organic C was higher in litter, so that ECM fungi have more potential resources, while a net gain of increased priming activity could still prevail.
Although laboratory studies suggest hyphal turnover to be rapid (once per week), turnover rate estimates for ECM hyphae in natural systems range from once per week to several months (Ekblad et al. 2013). Hence, albeit theoretically possible, ^{13}C acquisition of saprotrophic microbes by feeding on EM hyphal necromass (Phillips et al. 2012) seems unlikely within the short period between start of ^{13}C-CO_2 pulse labelling and harvest.

Relationship between ectomycorrhizal hyphae and associated bacteria

^{15}N addition had a significant effect on microbial community structure and the biomass of certain bacterial groups. While fungi and Gram negative bacteria prevailed constant, Actinobacteria and other Gram positive bacteria strongly declined in the compartments where N was added (Fig. 2.8). Furthermore, this loss of in biomass was not restricted to the compartment directly receiving labile N inputs (litter compartment), but occurred in the adjacent soil compartment as well. This response seems to be under fungal control, as the only theoretical connection between litter and soil compartments were fungal hyphae (the validity of the mesh acting as a barrier for diffusion or mass flow is demonstrated by the vast difference in $\delta^{15}N$ between litter and bulk soil; Fig. 2.13).

We argue that as ECM fungi were abruptly in a situation of easily available N in excess, their relationship to associated bacteria may have changed from cooperative to competitive. As a consequence, ECM fungi may have taken up the main part of the ^{15}N, allocating it towards plant roots, while impairing bacterial growth. The latter could have happened in different ways, for example by reducing the input of labile C, which may be vital for parts of the bacterial community, or by actively employing allelopathic strategies.

Indeed, our observations suggest that ECM fungi reduced the exudation of ^{13}C in the litter compartment: There was a significant decline in the absolute amount of ^{13}C incorporated into Actinobacteria and other Gram positive bacteria. Also, bacteria specific PLFAs tended to decline in relative incorporation of ^{13}C with N addition, whereas ^{13}C enrichment in DOC stayed constant.

It has been hypothesised that ECM antibiotics may be used in competition against saprotrophic fungi (Fernandez & Kennedy 2015). Accordingly, it would seem a viable strategy of ECM fungi to produce antibiotics against competing free living saprotrophs (fungal as well as bacterial) when practical. Indeed, fungi are known to produce a myriad of bacterial and fungal antibiotics (Keller et al. 2005), which may be utilized against pathogens, as well as in competitive situations. Furthermore, bacteria of the genus *Streptomyces* have been found to live in close association with ECM fungi (Seipke et al. 2012). *Streptomyces* is the largest antibiotic-producing bacterial genus known (Watve et al. 2017), and members of it promote mycelial growth (Schrey et al. 2005).

The relationship between ECM fungi and soil microbes associated with extraradical mycelium, however, is poorly understood. Based on our observations, the symbiosis seems on a 'razors edge', tilting between mutualistic and parasitic interactions depending on environmental conditions. In such an opportunistic mutualism, fungi would arguably occupy the more sustainable position, being able to control C flow to bacteria, as well as control bacterial growth by active exudation of antibiotic compounds.

Reciprocity in the ectomycorrhizal symbiosis

Here, we investigated potential plant-control on reciprocal C-for-N exchange at two spatial scales of the root system architecture. To achieve this, we (a) split the root system of young ECM *F. sylvatica* trees in half, and provided ECM fungi associated with only one half with easily available N, and (b) divided the roots of the latter half into smaller pieces (fourth- to fifth-order roots). If the plant could adjust its C transfer corresponding to fungal N delivery, the part of the root system which receives more N would also receive more C.

We report no control on nutrient exchange at a bulk root scale, that is, dividing the root system in half. In contrast, comparing smaller root pieces of only the N-amended side, we detect trends to reciprocity. This observation, however, still encompasses ambiguity, fitting weakly to a linear relationship (Fig. 2.15). Photosynthetically acquired ^{13}C shows both low and high abundance in roots, independently of ^{15}N transport; furthermore, ^{15}N is allocated only at high levels of ^{13}C. Hence, at this level of the root architecture (Fig. 2.15), our observations suggest the following:

First, the plant host has a low control on C transport, because abundance of recently photoassimilated ^{13}C never reached zero. As plants are generally not C-limited, unconditional belowground C transport might seem a viable strategy, while still achieving a net benefit by maintaining ECM.

Secondly, the delivery of larger amounts of N could also trigger plant-response to higher C allocation, because root pieces which received small to nil amounts of ^{15}N-labelled compounds might still have provided N from unlabelled sources. Indeed, only a part of the ECM fungal repertoire might have had access to the litter compartment, depending on their morphology, foraging strategies or exploration types. However, correlation with C and N per g dry root shows no relationship (Fig. S1). Plant control on the amount of C allocated does not necessarily contradict unconditional C transport, because a minimum amount of C might still be substantial for the fungus.

Indeed, control on C allocation to punish fungal partners which retain N is likely to have evolved as a mechanism to maintain mutualistic stability (Kiers & van der Heijden 2006). The overall nature of a close symbiotic relationship like mycorrhiza makes the emergence of cheating partners, which gain advantages via either selective opportunities or long-term defection from mutualism, highly probable in an evolutionary context (Douglas 2008). This may give room for parasitic interactions at a small scale, where ECM fungi hold back N but receive C, even though the net benefit for plant fitness by maintaining ECM stays positive.

Thirdly, a threshold for fungal N reward, based on plant C allocation, because high N delivery is always accompanied by high C allocation. Biological market theory implies that if cooperation from either partner is violated, the other should react with selective pressure by stopping nutrient transfer (Noë & Hammerstein 1995, Hammerstein & Noë 2016). As N is also a limiting resource for the fungus, a strong control on N delivery based on C offered by the plant seems a viable strategy.

However, our observations on reciprocal exchange are impaired by only looking at an instant situation. N sources might not be accessible to an ECM fungus at one point in time; nonetheless, the plant will keep allocating C to the fungus. Given a 'snapshot' observation, the fungus might consequently be distinguished as non-cooperative. However, the plant's investment will most likely pay off in the long run, when ECM hyphae reach new soil areas for N acquisition. This is conceptually equivalent to a repeated prisoner's dilemma, where conflict between interacting agents can be resolved in favour of reciprocity when trust in cooperation is established and encounters are recurring.

Conclusion

The mechanisms governing resource exchange between plants, mycorrhizal fungi and hyphae-associated bacteria remain a great unknown in mycorrhizal ecology, and indeed one of its most interesting aspects. Our results suggest distinct controls of C for N trading at both, the plant-fungus and the fungus-bacteria interface. Regarding the plant-fungus interface, our data hints to reciprocal exchange with a higher degree of control by the fungus. However, this does not exclude possible adjustments of C transport by the plant. In fact, many-to-many agent based interactions, as is the case in the mycorrhizal symbiosis (i.e. plants associate with several fungi, and fungi associate with several plants), are likely reciprocally controlled, thus maintaining evolutionary stability (Kiers & van der Heijden 2006). At the fungus-bacteria interface we found that ECM fungi transferred substantial amounts of the C they receive from their host plants to the hyphae-associated bacterial community. However, an increase in available N abruptly changes fungal-bacterial interactions from mutualistic to competitive, which could have happened because (i) C allocation to bacteria was reduced and (ii) production of antibiotic compounds decreased bacterial biomass. Taken together our results demonstrate that C and N flow through the plant-soil system is mediated by distinct controls on resource exchange not only between plants and their mycorrhizal partner, but also between mycorrhizal fungi and soil bacteria.

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Supplementary material

Table S1 Comparison of total root dry mass of labelled plants between the two soil compartments. The 'split-root' setup did not work as smoothly as anticipated, as root biomass varies strongly between the two soil compartments. Box 17 was omitted in most of the analysis.

Plant box	-N (g dry mass)	+N (g dry mass)	Ratio -N/+N
4	0.82	2.65	0.31
8	0.82	1.26	0.65
14	1.19	1.57	0.76
16	1.37	0.22	6.33
17	2.03	0.05	42.23
18	1.00	1.74	0.57
28	0.29	0.90	0.32
29	0.88	0.22	4.00

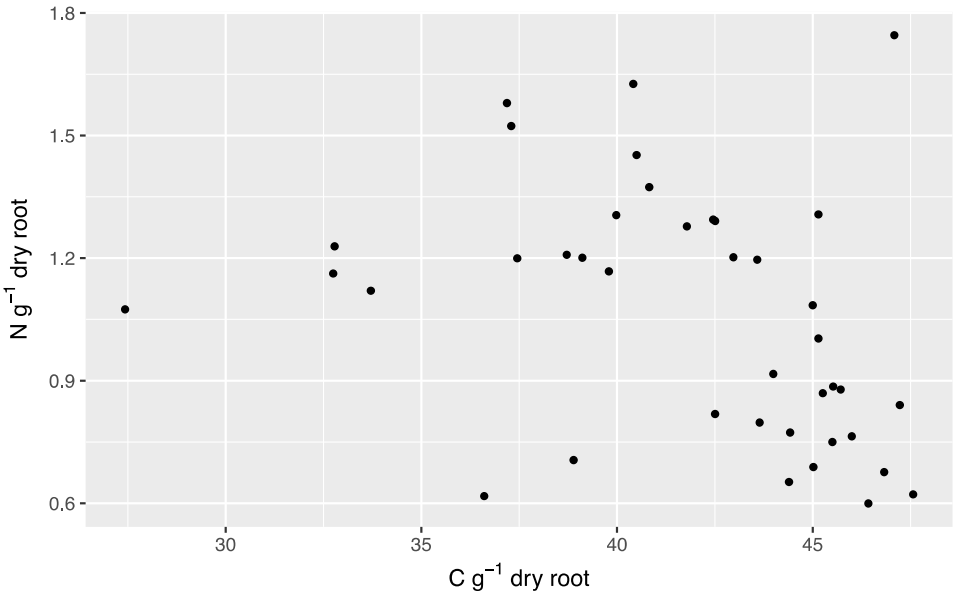

Figure S1 Correlation with C and N in individual root pieces of the ^{15}N-amended side. Each bulk root was separated into smaller root pieces based on root system architecture (3-4 pieces root^{-1}).

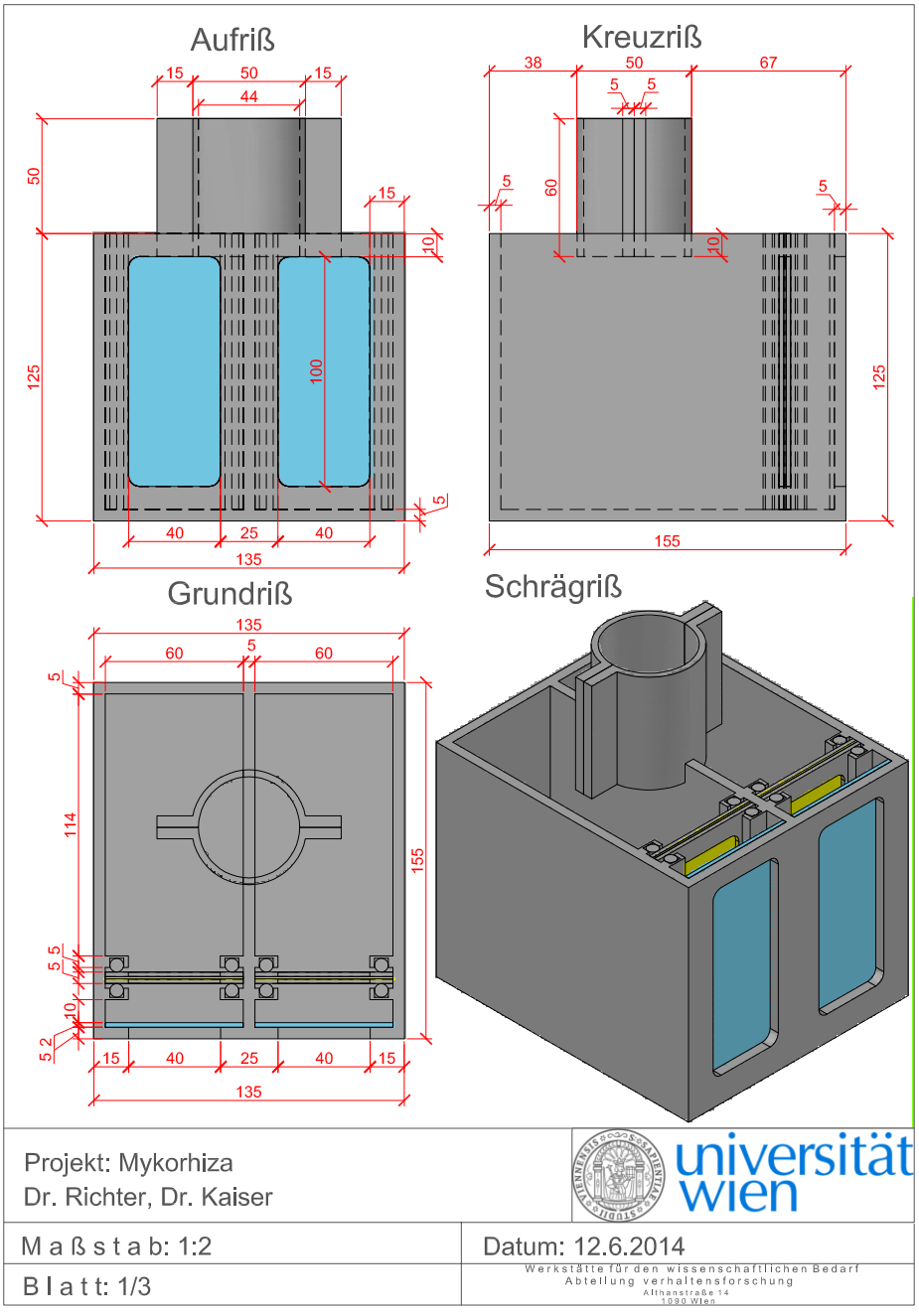

Figure S2 Dimensions and design of the split root boxes. Scale = 1:3, measurements in mm.

3. SUMMARY

Summary

The term mycorrhiza refers to the mutualistic relationship between plant roots and fungi, in which plants transfer photosynthetically fixed carbon (C) to associated mycorrhizal fungi, which provide nutrients and water to their plant host in return. Facilitating the colonisation of terrestrial habitats more than 400 million years ago, almost all higher plant species today rely on mycorrhizal fungi for their nutrition, which in turn interact with free-living soil saprotrophs. This work focuses on two aspects of resource exchange between plants, mycorrhizal fungi and soil saprotrophs.

First, we investigated resource exchange between plants and mycorrhizal fungi. In general, the mechanisms governing the relationship between plant and mycorrhizal fungi and thus the stability of the mycorrhizal symbiosis are poorly understood. Plants are typically associated with several mycorrhizal fungi, that generally show low host specificity, potentially forming associations with several, phylogenetically distant plants. Due to this capacity to interact with multiple partners, the emergence of cheating strategies by withholding resources is likely. Although the exchange of resources is considered mutually beneficial, the extent to which their provision depends on the mutual supply from the other partner is unknown. A reciprocal control of exchange rates has been demonstrated in arbuscular mycorrhizal systems, but the situation remains equivocal for the ectomycorrhizal (ECM) symbiosis.

Second, this study focuses on the process of nutrient acquisition via mycorrhizal hyphae in interaction with associated soil bacteria. Exudation of recent photosynthates into adjacent soil has frequently been shown to accelerate microbial soil organic matter decomposition, which in turn increases nutrient availability in the surrounding of the root. Referred to as 'rhizosphere priming effect', this input of labile C compounds by roots may alleviate the prevailing C limitation of saprotrophic microorganisms and increases their metabolic capacity to produce extracellular enzymes degrading complex organic compounds. This may result in an increased availability of essential nutrients for both microorganisms and plant. As mycorrhizal fungi usually have reduced capabilities to produce extracellular enzymes for organic matter decomposition compared to many free-living saprotrophs, analogous mechanisms may take place in the vicinity of mycorrhizal hyphae, the hyphosphere: Photosynthetically acquired C could potentially be exuded by mycorrhizal hyphae, facilitating supply of nutrients from root-distant soil areas for the fungus, and consequently its host plant. In this regard, the mycorrhizal symbiosis may conceptually be extended to interactions between mycorrhizal fungal hyphae and soil bacteria. While mycorrhizal transfer of plant-derived C to free living soil bacteria has been observed in arbuscular mycorrhizal systems, there is no evidence so far for ECM systems.

In this study we hypothesized (i) that reciprocal reward within the ECM symbiosis may be observed at the level of root system architecture, i.e., that plants allocate C preferentially to parts of their root system that receive more N by ECM fungi, (ii) that ECM fungi allocate recent photosynthates to associated hyphosphere bacteria, and (iii) that this allocation of plant-borne C is influenced by N availability.

To address these hypotheses, we conducted a split-root experiment with ectomycorrhizal *Fagus sylvatica* trees. Each plant was transferred from the field to a 'split-root'-box, dividing its root system into two parts, with each part growing into one of two disconnected soil compartments. Each of the two soil compartments was connected to a separated litter compartment by a mesh (35 µm) penetrable only for fungal hyphae, but not for roots. Stable isotope tracing was used to determine the fate of recent photosynthates and nutrients in this system, by exposing aboveground plants to a pulse of ^{13}C-CO_2, and applying ^{15}N-labelled ammonium and amino acids to one of the two litter compartments.

We subsequently compared ^{15}N-amended and untreated root system parts regarding their ^{13}C allocation, and traced the fate of recently photoassimilated ^{13}C into soil bacteria via phospholipid fatty acid stable isotope probing and nanoscale secondary ion mass spectrometry (NanoSIMS).

Our results show bidirectional C for N transport of ^{13}C photoassimilated by the plant and ^{15}N taken up by mycorrhizal hyphae from the litter compartment on short time scales. No plant control on this exchange was found at a bulk root scale, that is, no difference in ^{13}C enrichment between the two root system halves of one plant that have and have not received additional N. However, we detect some implications on potential reciprocity when dividing roots into smaller pieces: (i) plant C flux is unconditional, as ^{13}C-enrichment of root pieces were independent of fungal N delivery, with high ^{13}C-enrichments also occurring with little or no fungal N supply. (ii) On the contrary, fungal N delivery (high ^{15}N enrichment) was always linked to a high flow of recent photosynthates to the respective root pieces.

Our results show a rapid allocation of recent photosynthates via ECM hyphae to associated soil bacteria areas distant of the root (i.e. in hyphae-only litter compartments), with already significant enrichments in PLFAs specific for bacteria 17-24 hours after ^{13}C-CO_2 labelling. This provides support for a priming effect in an ECM hyphosphere. Hyphal exudation of labile C could facilitate metabolic activity and growth of hyphae-associated saprotrophic bacteria, accelerating organic matter degradation and in turn nutrient availability.

Contrary to previous observations, which found plant-derived labile C to be preferentially utilized by Gram negative bacteria in the rhizosphere, we find that most ^{13}C was allocated to Actinobacteria and other Gram positive bacteria.

We found a strong decline in biomass of Actinobacteria and other Gram-positive bacteria with N addition. This seems to be a reaction of ECM fungi acting on associated soil bacteria, as the effect was also observed in soil compartments where fertilized N would only be available for bacteria through hyphal transport. This effect was strongest in bacterial groups which also received most ^{13}C. We argue that because 'primed' bacteria are potentially N limited, an additional sudden input of easily available N sources would strongly perturb the relationship between ECM fungi and associated bacteria, putting them in a competitive situation. As a response, ECM fungi could (i) halt C exudation (supported by a decrease of ^{13}C in bacteria-specific PLFAs, but unchanged ^{13}C-enrichment in DOC), and/or (ii) produce antibiotic compounds, reducing saprotrophic growth.

Overall, our results suggest a high control of ECM fungi on N delivery based on the amount of C received by the plant, but conversely a low control of the plant on C delivery. They further support priming of saprotrophic bacteria via ectomycorrhizal hyphae as a potential mechanism to increase access to nutrients from soil organic matter for ectomycorrhizal fungi and their host plants. Our results however also indicate that hyphosphere priming may be a highly controlled process with hyphal exudation being able to rapidly respond to changing soil nutrient availabilities.

Zusammenfassung

Der Begriff Mykorrhiza bezeichnet die mutualistische Beziehung zwischen Pflanzenwurzeln und Pilzen, bei der Pflanzen durch die Photosynthese gewonnenen Kohlenstoff (C) an assoziierte Mykorrhizapilze weitergeben, die wiederum Nährstoffe und Wasser an ihren Wirt liefern. Die Besiedlung terrestrischer Lebensräume vor 400 Millionen Jahren wurde durch die Vergesellschaftung mit solchen Pilzen ermöglicht. Dadurch sind fast alle rezenten Pflanzen in ihrer Nährstoffbeschaffung immer noch auf Mykorrhizapilze angewiesen, die wiederrum mit saprotrophischen Bodenmikroorganismen interagieren. Diese Arbeit beleuchtet zwei Aspekte dieses Resourcenaustausches zwischen Pflanze, Pilz und Bodenmikroorganismen.

Zum einen beschäftigt sich diese Arbeit mit der gegenseitigen Kontrolle des Resourcenaustausches zwischen Pflanze und Pilz. Generell weiss man sehr wenig über die Mechanismen, welche die Beziehung zwischen Pflanzen und Mykorrhizapilzen und somit die Stabilität der Mykorrhizasymbiose bestimmen. Pflanzen sind typischerweise mit mehreren Mykorrhizapilzen assoziiert, die wiederrum Assoziationen mit mehreren phylogenetisch nicht nahe verwandten Pflanzen bilden. Aufgrund dieser Fähigkeit, mit mehreren Partnern zu interagieren, ist die Entstehung von Betrugsstrategien wahrscheinlich, in denen Ressourcen zurückgehalten werden. Obwohl der Austausch von Ressourcen als für beide Seiten vorteilhaft angesehen wird, ist das Ausmaß, in dem ihre Bereitstellung von der gegenseitigen Versorgung durch den anderen Partner abhängt, unbekannt. Während eine gegenseitige Kontrolle des Austausches von C und Nährstoffen in arbuskulären Mykorrhizasystemen bereits gezeigt wurde, bleibt die Situation für die Ektomykorrhiza (ECM) Symbiose unklar.

Zum anderen konzentriert sich diese Studie auf die Wechselwirkung von Mykorrhizahyphen mit assoziierten Bodenbakterien. Experimente haben bereits mehrmals demonstriert, dass Wurzelexsudation von frisch photosynthetisiertem C den mikrobiellen Abbau von organischer Substanz im Boden beschleunigt, was wiederum die Nährstoffverfügbarkeit in der Umgebung der Wurzel erhöht. Dieser Mechanismus wird als "Rhizosphären-Priming-Effekt" bezeichnet. Der Eintrag von labilen C-Verbindungen durch Wurzeln reduziert die C-Limitierung von saprotrophen Mikroorganismen, und erhöht ihre metabolische Kapazität, extrazelluläre Enzyme zu erzeugen, welche komplexe organische Verbindungen abbauen. Dies kann zu einer erhöhten Verfügbarkeit von essentiellen Nährstoffen sowohl für Mikroorganismen als auch für Pflanzen führen. Da Mykorrhizapilze im Vergleich zu saprotrophen Pilzen normalerweise verminderte Fähigkeiten zur Produktion von extrazellulären Enzymen für die Zersetzung organischer Substanzen haben, könnten analoge Mechanismen in der Umgebung von Mykorrhizapilzen durch Exsudationen von labilem C durch Hyphen auftreten. In dieser Hinsicht kann die Mykorrhizasymbiose konzeptionell auf Wechselwirkungen zwischen Mykorrhiza-Pilzhyphen und Bodenbakterien erweitert werden. Während in arbuskulären Mykorrhizasystemen ein Transfer von kürzlich photosynthetisiertem C auf Bodenbakterien der Hyphosphäre beobachtet wurde, gibt es bisher keine Beweise für ECM-Systeme.

Unsere Hypothesen waren, (i) dass eine gegenseitige Belohnung innerhalb der ECM-Symbiose auf der Ebene der Wurzelsystemarchitektur stattfindet (d.h. dass Pflanzen C bevorzugt in Teile ihres Wurzelsystems leiten, die mehr N durch ECM-Pilze erhalten), (ii) dass ECM-Pilze kürzlich photooxidierten C an

assoziierten Hyphosphärenbakterien weitergeben, und (iii) dass diese Weitergabe von C durch N-Verfügbarkeit beeinflusst wird.
Um diesen Hypothesen zu testen, führten wir ein ‚Split-Root'-Experiment mit jungen Buchen (*Fagus sylvatica*) durch. Jede Pflanze wurde direkt aus ihrer natürlichen Umgebung in eine ‚Split-Root'-Box überführt, wobei ihr Wurzelsystem in zwei Teile geteilt wurde, und jeder Teil in eine von zwei getrennten Bodenkammern gepflanzt wurde. Diese Bodenkammern waren jeweils durch ein feinmaschiges Netz (35 µm), das nur für Pilzhyphen, aber nicht für Wurzeln durchlässig ist, mit einer getrennten Kammer verbunden, in der sich Pflanzenstreu befand. Um C und N Flüsse in diesem System zu bestimmen wurden oberirdische Pflanzenteile einer ^{13}C-CO_2–angereicherten Atmosphäre ausgesetzt, und eine Lösung mit ^{15}N-markierten Ammonium und Aminosäuren nur einer der beiden Pflanzenstreu-Kammern zugesetzt.
Wir analysierten anschließend die ^{13}C-Verteilung in den beiden Wurzelsystemhälften, von denen nur jeweils einer Zugang zu ^{15}N hatte, und verfolgten den Fluss von kürzlich photoassimiliertem ^{13}C in Bodenbakterien mittels Analyse von stabilen Isotopen in Phospholipid-Fettsäuren und Nano Sekundärionen-Massenspektrometrie (NanoSIMS).
Unsere Ergebnisse zeigen bidirektionalen Transport von kürzlich photoassimilierten ^{13}C und durch Mykorrhizahyphen aus den Pflanzenstreu-Kammern aufgenommenen ^{15}N. Offensichtlich gab es keine Kontrolle der Pflanze über diesen Austausch, da wir keine Unterschiede in der ^{13}C-Anreicherung zwischen den zwei Wurzelsystemhälften einer Pflanze gefunden haben. Wir fanden aber einige Implikationen, die auf eine mögliche gegenseitige Kontrolle des Resourcenaustausches hinweisen, als wir Wurzelhälften in kleinere Stücke teilten: (i) Pflanzen geben C unabhängig von der Menge des erhaltenen N an Mykorrhizapilze ab, was dadurch gezeigt wurde dass ^{13}C-Anreicherungen von Wurzelstücken unabhängig von der N-Freisetzung des Pilzes waren. (ii) Im Gegensatz dazu war die N-Abgabe von Pilzen immer mit einem hohen C-Fluss an die jeweiligen Wurzelstücke verbunden.
Unsere Ergebnisse zeigen eine schnelle Weitergabe von kürzlich photoassimiliertem C über ECM-Hyphen zu assoziierten Bodenbakterien in Bereichen welche nicht für Wurzeln zugänglich waren (d.h. in Pflanzenstreukammern, zu denen nur Hyphen Zugang hatten), sichtbar durch bereits signifikante ^{13}C Anreicherungen in bakterienspezifischen PLFAs nur 17-24 Stunden nach der Photoassimilierung von mit ^{13}C angereichertem CO_2. Diese Beobachtung spricht für einen ‚Priming-Effekt' in der Hyphosphäre von ECM-Pilzen. Die Exsudation von labilem C über Mycorrhizahyphen könnte die metabolische Aktivität und das Wachstum von Hyphen-assoziierten saprotrophen Bakterien erleichtern, und damit den Abbau organischer Substanz und die Verfügbarkeit von Nährstoffen begünstigen.
Im Gegensatz zu Beobachtungen aus anderen Experimenten, bei denen durch Pflanzen ausgeschiedener labiler C bevorzugt von Gram-negativen Bakterien genutzt wurde, nahmen in unserem Experiment vor allem Actinobakterien und andere Gram-positive Bakterien ^{13}C auf.
Die N Zugabe löste einen starken Biomasseverlust von Actinobakterien und anderen Gram-positiven Bakterien in unserem Experiment aus. Dieser Effekt wurde nicht nur in den Pflanzenstreukammern sondern auch in den anschliessenden Bodenkammern beobachtet, in welche keine nennenswerten Mengen N transportiert wurden. Das deutet darauf hin, dass es sich dabei um

einen negativen Effekt von ECM-Pilzen auf Bodenbakterien als Reaktion auf erhöhte N Verfügbarkeit handelt.
Dieser Effekt war am stärksten in bakteriellen Gruppen, die auch am meisten ^{13}C erhielten. Dementsprechend schlussfolgern wir, dass, da ‚geprimte' Bakterien potentiell N-limitiert sind, eine zusätzliche plötzliche Verfügbarkeit von labilem N die Beziehung zwischen ECM-Pilzen und assoziierten Bakterien stark perturbieren, und sie damit in eine Konkurrenzsituation versetzen würde. Als eine Reaktion könnten ECM-Pilze (i) die C-Exsudation stoppen (experimentell unterstützt durch eine Abnahme von ^{13}C in bakterienspezifischen PLFAs, mit unveränderter ^{13}C-Anreicherung in DOC) und/oder (ii) Antibiotika produzieren, wodurch das Wachstum saprotropher Mikroorganismen verringert wird.
Insgesamt deuten unsere Ergebnisse auf eine starke Kontrolle von ECM-Pilzen auf die pflanzliche N-Versorgung hin, aber umgekehrt eine geringe Kontrolle der Pflanze auf C-Fluss. Unsere Ergebnisse unterstützen die Hypothese dass Ektomykorrhiza-Hyphen zum ‚Priming' von saprotrophen Bakterien und damit zur Erhöhung der Nährstoffverfügbarkeit aus organischer Bodensubstanz für Ektomykorrhizapilze und ihre Wirtspflanzen beitragen. Unsere Ergebnisse deuten jedoch auch darauf hin, dass die Stimulierung von Mikroorganismen in der Hyphosphäre ein hochgradig kontrollierter Prozess ist, bei dem Hyphenexudation schnell auf sich ändernde Bodennährstoffverfügbarkeiten reagiert.